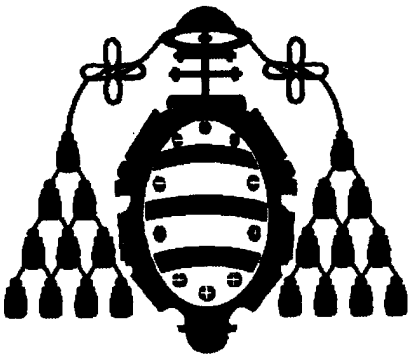

UNIVERSIDAD DE OVIEDO

Departamento de Biología de Organismos y Sistemas

Programa de Doctorado: "Biología aplicada a la sostenibilidad de recursos naturales (Mención de calidad)"

" Desplazamientos altitudinales y características ecológicas de los abejorros (*Bombus* spp.) de la Cordillera Cantábrica"

"Elevational shifts and ecological traits of bumblebee (*Bombus* spp.) assemblages of the Cantabrian Range"

TESIS DOCTORAL

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Oviedo 2013

RESUMEN DEL CONTENIDO DE TESIS DOCTORAL

1.- Título de la Tesis	
Español/Otro Idioma: Desplazamientos altitudinales y características ecológicas de los abejorros (*Bombus* spp.) de la Cordillera Cantábrica	Inglés: Elevational shifts and ecological traits of bumblebee (*Bombus* spp.) assemblages of the Cantabrian Range

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RESUMEN (en español)

El impacto negativo de la actividad humana sobre los ecosistemas terrestres alcanza proporciones globales. En concreto, la pérdida de hábitat y el cambio de los patrones climáticos constituyen dos de las principales amenazas para la biodiversidad que éstos albergan. Como consecuencia de los actuales cambios ambientales, las especies pueden experimentar cambios drásticos en sus rangos de distribución geográfica y abundancia. Por otra parte, otros factores ambientales como la disponibilidad de hábitat e interacciones bióticas también podrían determinar los rangos de distribución de las especies. Sin embargo, las distintas especies difieren en la magnitud y dirección de la respuesta a cambios ambientales. Se considera que la respuesta diferencial de las distintas especies puede conducir a importantes cambios en la estructura y composición de las comunidades ecológicas, así como en las interacciones entre especies.

Los abejorros (*Bombus* spp.) son insectos polinizadores particularmente importantes en climas fríos así como en sistemas montañosos. Las poblaciones de abejorros, al igual que otros polinizadores, están sufriendo declives a escala global; se considera que las causas del declive generalizado de abejorros están relacionadas con cambios en el uso del suelo que afectan a la disponibilidad de recursos florales. Por otra parte, el cambio climático podría tener un papel importante en el declive de los abejorros, ya que se ha propuesto que las especies con menor tolerancia climática serían más susceptibles al declive. La Cordillera Cantábrica es una región del noroeste de España que alberga una elevada diversidad de especies de abejorros y constituye el límite sur del área de distribución de algunas de ellas. El objetivo principal de esta tesis consiste en determinar si han tenido lugar cambios en la distribución y abundancia relativa de las especies de abejorros en un sistema montañoso en un periodo de 20 años. Por otro lado, se pretende determinar qué características ecológicas de las especies determinan los cambios observados así como evaluar el papel de variables climáticas y topográficas en los cambios observados. Por último, se llevó a cabo la cuantificación de nicho trófico de especies de abejorros a lo largo del gradiente altitudinal.

La mayoría de las especies han desplazado sus límites de distribución inferior o superior hacia zonas más altas, resultando en reducciones considerables en el rango de altitud en el que aparecen. La respuesta de las distintas especies difirió en la magnitud y dirección de la misma. En conjunto, estos efectos dieron lugar a la homogenización de las comunidades de abejorros entre altitudes medias y zonas altas. La tolerancia climática de las distintas especies determinó en gran parte la magnitud de su respuesta. En concreto, las especies con mayor amplitud de nicho climático mostraron cambios más pronunciados en las condiciones climáticas de un periodo respecto al anterior, así como una mayor pérdida en el rango de altitud ocupado. La distribución y abundancia de las especies de abejorros en la Cordillera Cantábrica está determinada fundamentalmente por variables climáticas (especialmente temperatura). Las distintas especies de abejorros presentan nichos tróficos más restringidos y un mayor solapamiento en el uso de recursos en comunidades con mayor riqueza de especies. Este resultado sugiere dietas más especializadas y competencia interespecifica más intensa en dichas comunidades.

Los resultados obtenidos proporcionan una visión más amplia sobre la respuesta de los abejorros a cambios ambientales en sistemas montañosos. Además, el estudio de abejorros a lo largo de un gradiente de altitud constituye una herramienta especialmente útil para evaluar el impacto del cambio global en ecosistemas de montaña. Por último, los patrones y respuestas descritos podrían tener aplicaciones en conservación, ya que permiten predecir la vulnerabilidad de las especies de abejorros en un contexto de cambio global.

RESUMEN (en Inglés)

The deleterious impact of human activities on terrestrial biodiversity has reached global proportions. Climate change, together with habitat loss and overexploitation, has become one of the most important drivers to biodiversity losses. One important potential response to such climatic-related-environmental changes is that species track their climate-envelope, altering the patterns of species distributions and abundance. However, other environmental factors such as the availability of suitable habitats and food resources as well as interactions with other species might equally define each species' distributions. Consistent with this, there is considerable interspecific variation in both the direction and the magnitude of species' response to environmental changes. These species-specific responses could further disrupt species interactions and lead to cumulative changes on community structure and composition.

Bumblebees (*Bombus* spp.) are major pollinators in cold environments and mountains. Like many other pollinators, bumblebees are experiencing an ongoing worldwide decline, which has been associated to different components of global change, such as changes in land use. However, climate warming might have a disproportionate effect in this case since bumblebee vulnerability has been linked to the climatic specialization of these declining species. The Cantabrian Range (NW Spain) has an exceptionally high diversity of bumblebee species and constitutes the southern limit of distribution for many of them. This thesis focuses on the potential changes in distribution and abundance of bumblebee species in a mountain range over a period of 20 years. In addition, we endeavoured to ascertain whether these changes could be explained by species-specific traits and evaluated the potential effect of climate change and land cover on the changes observed. Finally, we carried out the quantification of bumblebee trophic niches along the elevational gradient.

Most species shifted their upper- or lower-elevation boundaries upwards, resulting in narrower elevational ranges from the first to the second study period. Species' responses were species-specific and resulted in the homogenization of bumblebee assemblages, especially between medium and upper elevations. Climatic tolerance mainly determined each individual species' response. Specifically, bumblebee species with broad climatic tolerance showed a reduction in their elevational range and greater changes in the climatic conditions at which they occurred from one period to another. Bumblebee presence and abundance patterns in the Cantabrian Range were found to be determined by climatic variables (notably temperature). Bumblebee species showed narrower trophic niches and a larger degree of niche overlap between species pairs in species-rich assemblages, suggesting a more specialized diet and stronger competition in those communities.

The results presented herein provide increased knowledge about the response to environmental change of bumblebees in mountain systems. Furthermore, we provide evidence of the suitability of bumblebee species in mountain systems as an ecological framework for testing the impact of global change. The patterns and processes described have applied implications for conservation as they allow the prediction of vulnerability of bumblebee species given the susceptibility in the face of global change.

Durante la realización de esta Tesis Doctoral he disfrutado de una beca del Programa "Severo Ochoa" de ayudas predoctorales para la formación en investigación y docencia del Principado de Asturias (ref. BP08-047).

Este trabajo estuvo financiado por el proyecto CGL2009-11302 del Ministerio de Ciencia e Innovación, Gobierno de España.

La investigación presentada en esta Tesis Doctoral se ha desarrollado en el Departamento de Biología de Organismos y Sistemas de la Universidad de Oviedo.

CONTENTS/CONTENIDOS

AGRADECIMIENTOS

Casi todas las tesis suelen ser, en general, un largo proceso. Para mí, el mejor recuerdo será sin duda el de todas las personas que he ido conociendo a lo largo de este periodo. He aprendido mucho de todos vosotros, no sólo en lo profesional sino también en lo personal. Desde luego, no habría llegado hasta aquí sin vuestra ayuda y apoyo constante; así que esta tesis también es en parte vuestra.

En primer lugar, mi director de tesis, Moncho Obeso, me dio la oportunidad de dar los primeros pasos en la investigación. Más tarde, confió en mí para llevar a cabo este proyecto, que espero haber desarrollado con éxito. Tengo mucho que agradecerle: desde los primeros días de campo hasta sus brillantes ideas y explicaciones, pasando por toda la ayuda que me ha prestado para poder desarrollar esta tesis. Y por qué no, también por redescubrirme unos bichos peludos y fascinantes que, sinceramente, nunca imaginé que darían para tanto.

José M. Herrera, mi codirector. Su capacidad de trabajo y de implicarse completamente en el proyecto todavía me asombran; sin duda, su incorporación supuso una mejora sustancial en la calidad del trabajo. Y por supuesto, la paciencia infinita para explicarme y ayudarme a desarrollar los manuscritos y el resto de la tesis. Y por los ánimos, hasta el final.

Si no fuera por Amalia Segura, a estas alturas probablemente estaría todavía moliendo y encapsulando muestras para análisis de isótopos estables. Amalina, ¡no sé por donde empezar! Me has acompañado al campo, siempre de buen humor y con ganas de trabajar a pesar del cansancio. Todavía me río al recordarte durmiendo en medio del brezal. Y todo el trabajo de laboratorio: has pesado, molido, encapsulado y vuelto a pesar todo lo que se me iba ocurriendo ¿Qué habría hecho sin tu ayuda y eficacia?. Lo mejor de todo, la sonrisa y el constante optimismo ¡Gracias!

Con Joaquina, mi "compi" de tesis, he compartido casi todos los pasos de esta tesis (campo, sala, cursos de doctorado, congresos…). El primer verano de

campo fue especialmente duro: creo que nunca en mi vida he pasado tanto frío como en La Farrapona ni tanto calor como en Las Señales. Pero también fue divertido, y desde luego muy variado. Parcelas, orugas, polinizadores, bichos en general … Siempre que he tenido dudas de cualquier tipo las he compartido contigo y me has ayudado. Tu amistad y compañía han hecho mucho más llevadera esta tesis.

El resto de Profesores, investigadores, becarios y técnicos del área han contribuido a hacer que, en general, todo fuera más fácil, ya sea ayudándome directamente o generando buen ambiente.

Mario Quevedo no sólo compartió conmigo sus conocimientos e impresiones sobre ciencia, *wilderness* y (quizás más en particular) isótopos estables. Desde el primer momento fue especialmente sincero advirtiéndome de donde me metía, aunque yo no lo entendiera muy bien, y sobre todo para que no me pasara al "lado oscuro". Mariajo Bañuelos, siempre con buena disposición para explicar cualquier cosa, discutir sobre ciencia y también para charlar y reírse un poco, que nunca está de más. Paola Laiolo y Jordi Molla-Laraño siempre tuvieron un rato para explicarme cualquier duda (en general estadística), a pesar de estar bastante liados todo el tiempo; también se interesaban por mi trabajo y me animaron cuando las cosas parecían más difíciles. Gracias especiales a David Álvarez por la impresionante foto de la portada (quien haya intentado fotografiar un abejorro sabrá lo difícil que es porque … prácticamente nunca se están quietos) y a Alfredo G. Nicieza por prestarme los *data loggers* para el experimento de termorregulación.

Para mí, ha sido un placer y un lujazo compartir sala con Amalia, Joaquina, Nere, Alberto, Javi Naves, Ricardo, Sonia… y con todos los demás que han ido pasando en algún momento por esa misma sala (Xurde, Maite, Isa, Leandro, Gerardo, Ana Palmero, Valentine…), o por la sala contigua (María Morán, Dani M., Cris, Ali, Juan, Karlina, Nico, Bea, Leti, Vane, Magda, Susana, Antonella, Carlos C., Carlos G, Sonia, Fernando, Javi, Axa, Albert…).

A Nere, mil gracias por todo. Por las risas, las "cerves" después de yoga, por poder hablar de todo contigo, y en definitiva, por ser una buena amiga.

Dani M. y Cris, de los Stark y Targaryen, respectivamente. Menos mal que estabais en el departamento estos meses, cuando ya se habían marchado casi todos, siempre de buen humor y con ganas de reírse un rato entre tantas horas de trabajo.

Javi Rodríguez, gracias por los comentarios sobre los Capítulo 1 y 3 por otras tantas cuestiones relacionadas con esta tesis. Bea Blanco-Fontao, gracias por todos los consejos y explicaciones sobre metodología y análisis de isótopos estables.

Gracias a Jorge Sostres por su eficacia y disposición para solucionar cualquier problema, y porque también le tocó lo suyo de moler-pesar-encapsular.

Leonor Calvo por enseñarnos sus parcelas de *Callluna vulgaris*… que resultaron tener una diversidad enorme de abejorros.

Ronnie Lendrum corrigió la mayor parte del inglés de esta tesis y mejoró considerablemente el estilo de la misma ¡Muchas gracias!

Mi tío Rafa me construyó un "*bee fligth arena*" que, aunque no salga en esta tesis, fue indispensable para llevar a cabo un experimento de termorregulación en abejorros.

Durante el desarrollo de esta tesis he podido disfrutar de dos estancias breves de investigación. Gracias a Beverley Glover y su equipo (Matthew, Alison, Katrina, Rubén, Sam, Alison, Rachel, Murphy y Veronica) en la Universidad de Cambridge (Inglaterra) por darme la oportunidad de aprender a trabajar con colonias de abejorros en el laboratorio y por su buena acogida en general. En la Universidad de Stirling (Escocia) conocí a Dave Goulson, un gran investigador y naturalista, al que tengo que agradecer también su magnífico libro que me resolvió muchas dudas sobre la biología de los abejorros. Sus estudiantes me hicieron sentir como en casa, a pesar de la cruda primavera escocesa: Andreia Penado,

Hannah Feltham, Ciaran Ellis y Steph O'Connor. Y por supuesto mis compañeras del "*beautiful bright flat*" en Stirling: Hazel Batty, Angelica Palmegianni y Anne Chang.

A mis amigas: Naty, Patri, Lynnette, Ángela, Violeta, Leandra, María Martínez ... Gracias por estar siempre ahí, por escuchar y preocuparse. Gracias por todo los demás (especialmente esos domingos interminables). Sobran las palabras.

opacompañaste alguna vez a muestrear! Si no fuera por tí, probablemente no habría empezado esta gran aventura ¡Muchas gracias!

Mi gatina Donna, que se pasó horas durmiendo encima de mis rodillas mientras escribía, obligándome a no moverme de la silla.

Y por supuesto, a mis padres Javi y Cati, y mis hermanos Iyán y Elisa. No han sido fáciles los últimos meses, pero habéis sido cariñosos y compresivos más allá de lo esperable. Siempre me habéis apoyado y eso lo llevaré siempre conmigo. Al resto de mi familia también, por el cariño, ánimos e interés; aunque no entendieseis muy bien qué era lo que estaba haciendo y porqué tardaba tanto en escribir "*el llibru*". En especial, mi güelita Laros, que tiene "*tantas ganas de que acabe ya de estudiar*". Yo también, güeli, pero no tengo muy claro si eso se acaba algún día.

Espero no olvidarme de nadie. Si es así, a todos los que sientan que deberían estar aquí y no están ¡gracias a todos!

Resumen

RESUMEN

El impacto negativo de la actividad humana sobre los ecosistemas terrestres alcanza proporciones globales. En concreto, la pérdida de hábitat y el cambio de los patrones climáticos constituyen dos de las principales amenazas para la biodiversidad que estos albergan. Como consecuencia de los actuales cambios ambientales, las especies pueden experimentar cambios drásticos en sus rangos de distribución geográfica. Estos cambios incluyen la expansión de los márgenes de distribución de las especies hacia el norte o hacia zonas más altas, así como extinciones en el límite sur de su distribución. Sin embargo, las distintas especies difieren en la magnitud y dirección de la respuesta. Se considera que las especies con mayor capacidad de dispersión y mayor grado de generalización ecológica serían más susceptibles de colonizar nuevas regiones en un contexto de cambio global. Por otra parte, la respuesta diferencial de las distintas especies a los cambios ambientales puede conducir a importantes cambios en la estructura y composición de las comunidades ecológicas.

Los abejorros (*Bombus* spp.) son insectos polinizadores particularmente importantes en climas fríos así como en sistemas montañosos. Las poblaciones de abejorros, al igual que otros polinizadores, están sufriendo declives a escala global; se considera que las causas del declive generalizado de abejorros están relacionadas con cambios en el uso del suelo que afectan a la disponibilidad de recursos florales. Por otra parte, el cambio climático podría tener un papel importante en el declive de los abejorros, ya que se ha propuesto que las especies con menor tolerancia climática serían más susceptibles al declive.

La Cordillera Cantábrica es una región del noroeste de España que alberga una elevada diversidad de especies de abejorros y constituye el límite sur del área de distribución de algunas de ellas. El objetivo principal de esta tesis consiste en determinar si han tenido lugar cambios en la distribución de especies y comunidades de abejorros en la Cordillera Cantábrica en un periodo de 20 años,

así como identificar que características ecológicas de las especies determinan los cambios observados.

En el **capítulo 1** se examinan los cambios en el rango altitudinal y abundancia relativa de las especies de abejorros en un periodo 20 años. Para ello, en los años 2007-2009 se muestrearon localidades previamente muestreadas en 1988-1989. Se determinaron los cambios en los límites superior e inferior, altitud media y rango altitudinal, y se compararon las comunidades de abejorros localizadas en zonas altas y bajas y a altitudes medias para comprobar si los cambios a nivel de especie determinaron alteraciones a nivel de comunidad. Los resultados obtenidos indican que, en un periodo de 20 años, las especies de abejorros de la Cordillera Cantábrica han desplazado sus límites de distribución inferior o superior hacia zonas más altas, resultando en reducciones considerables en el rango de altitud en el que aparecen. Estos cambios se produjeron como resultado de procesos de extinción y colonización de las especies a lo largo del gradiente altitudinal así como cambios en la abundancia relativa de las mismas. Las extinciones fueron predominantes en zonas bajas y a altitudes medias, mientras que sólo las zonas altas presentaron un balance positivo de colonizaciones. En conjunto, estos efectos determinaron la homogenización de las comunidades de abejorros entre altitudes medias y zonas altas.

En el **capítulo 2** se investiga el papel de determinados rasgos ecológicos de las especies de abejorros en la respuesta especie-específica al cambio en los patrones de temperatura tras dos décadas en la Cordillera Cantábrica. Se relacionan rasgos ecológicos como el nicho climático o la capacidad dispersiva con el cambio en rango altitudinal o en las condiciones climáticas a las que las especies se encuentran en ambos periodos. Las especies con mayor amplitud de nicho climático mostraron cambios más pronunciados en las condiciones climáticas de un periodo respecto al anterior, así como una mayor pérdida en el rango altitudinal ocupado.

En el **capítulo 3** se evalúa el efecto de variables climáticas, topográficas y de configuración del hábitat a distintas escalas sobre la presencia y abundancia de

algunas especies de abejorros. Para ello se construyeron modelos predictivos que analizan el efecto de dichos factores a escalas sucesivamente mayores, empleando radios de 100 a 3000 m, con el fin de cubrir los rangos de la mayor parte de los desplazamientos que llevan a cabo los abejorros. La distribución y abundancia de las especies de abejorros en la Cordillera Cantábrica estuvo determinada fundamentalmente por variables climáticas. Por otra parte, los modelos predijeron una respuesta a cambios en variables climáticas en los cambios en altitud en la mayor parte de las especies analizadas, mientras que solamente tres especies respondieron a potenciales cambios en el paisaje.

En el **capítulo 4** se estima la amplitud de nicho trófico y el grado de superposición trófica entre las distintas especies de abejorros mediante relaciones isotópicas de ^{13}C y ^{15}N a lo largo del gradiente altitudinal. Las distintas especies de abejorros presentaron nichos tróficos más amplios y menor solapamiento en el uso de recursos en comunidades con menor riqueza de especies (localizadas a ambos extremos del gradiente de altitud). A altitudes medias (en torno a 1500 m), donde la diversidad y abundancia de abejorros alcanza el máximo en la Cordillera Cantábrica, las especies de abejorros mostraron nichos tróficos más especializados, así como un mayor solapamiento en el uso de recursos. Por otra parte, especies con longitud de probóscide similar mostraron un mayor solapamiento de nicho trófico. Estos resultados sugieren que la competencia interespecifica es importante a la hora de determinar la composición y abundancia de las comunidades de abejorros.

Los resultados obtenidos proporcionan una visión más amplia sobre la respuesta de los abejorros a cambios ambientales. Además, el estudio de abejorros a lo largo de un gradiente altitudinal constituye una herramienta especialmente útil para evaluar el impacto del cambio global en ecosistemas de montaña. Por último, los patrones y respuestas descritos tienen importantes consecuencias en conservación, ya que pueden permitir predecir respuestas e identificar que especies serían más vulnerables frente al cambio global.

General Introduction

GENERAL INTRODUCTION

Global change and evidence of species range shifts

The deleterious impact of human activities on terrestrial biodiversity is now sufficiently widespread that it has reached global proportions (Whalter et al. 2002, Root et al. 2003, Simmons and Thomas 2004, Bradshaw and Holzapfel 2006, Parmesan 2006). Fossil-fuel combustion and deforestation have increased the concentration of atmospheric carbon dioxide (CO_2) by almost 30% in the past three centuries and subsequently global surface temperature has warmed by *ca.* 0.74°C (IPCC 2007). Other abiotic factors that have also been altered by human activity include changes in precipitation patterns and extreme weather events, resulting in a general increase in climatic variability. Not surprisingly, climate change, together with habitat loss and overexploitation, has become one of the most important drivers to biodiversity losses at all organization levels and spatial scales (Vitousek 1994, Fahrig 2003, Thomas et al. 2004, Jetz et al. 2007).

One important potential response to such climate-related environmental changes is that species track their climate-envelope, altering patterns of species distribution and abundance (Tingley et al. 2009, Devictor et al. 2012). Changes in species spatial distribution include contractions at the warm range limits (extinction events) or expansions (colonizations) at the northern or upper elevational boundaries (Parmesan 2006). Although there is now ample evidence of range shifts towards higher elevations and latitudes for a wide range of plants and animals (Parmesan and Yohe 2003, Hickling et al. 2006, Lenoir et al. 2008), contractions at the warm, lower elevation and latitudinal margins remain understudied (but see Wilson et al. 2005, Chen et al. 2011*a*).

However, it is not only climatic conditions that define each species' distributions, but also other environmental factors such as the availability of suitable habitats and food resources as well as interactions with other species, all of which might hamper the probabilities of species colonizing new sites (Davis et

al. 1998). Thus, knowledge of the requirements of each species is fundamental to understanding the species' response and to predicting its future distributions in the face of global change (Easterling et al. 2000). Further to this, there is considerable interspecific variation in both the direction and the magnitude of geographical and elevational displacements, which suggests that individualistic traits might interact to modulate each species' realized response to climate warming (Schweiger et al. 2008, Buckley et al. 2010, Angert et al. 2011). In this sense, it has been suggested that species with greater dispersal ability, reproductive rate and ecological generalization should be more likely to expand into new regions under climate change (Lenoir et al. 2008, Pöyry et al. 2009, Angert et al. 2011).

The potential of the ecological niche as a predictive tool to evaluate species response to global change

The niche concept combines both the requirements of a species for existence in a given environment and its impact on that environment (Grinnell 1917, Elton 1927, Hutchinson 1957, Chase and Leibold 2003). Therefore, it is fundamental in determining not only species abundance and geographic distributions (Grinnell 1917), but also small-scale interactions (e.g., trophic interactions) and the role of a species in the community (Hutchinson 1957, 1978, Soberón 2007). The ecological niche should thus be one of the most powerful tools for evaluating the responses of organisms, populations and ecological communities to global change (Pulliam 2000, Chase and Leibold 2003).

In this sense, the climatic niche concept *sensu* Grinnell (1917) refers to the relationship between the climatic tolerances of a species and its geographic distribution, and might limit the ability of a species to colonize new areas. When environmental change occurs over short temporal and large spatial scales, species are expected to move geographically by tracking their climatic niches through time to remain in a favourable climate space (Wiens and Graham 2005, Tingley 2009).

Thus, this ecological trait should be a useful indicator of species response to climate change and able to be used to predict future distributions under different climatic scenarios (e.g., Peterson et al. 2002). However, biotic interactions also affects where a species reaches its limits (Hutchinson 1957, Davis 1998, Schweiger et al. 2008). The quantification and study of the ecological niche at local scale (e.g., the trophic niche) should reveal important consequences on interactions among coexisting species, and therefore predict changes in trophic structure and community dynamics.

Specialist species may be more vulnerable than versatile generalist if they are subject to rapid changing environmental conditions (Warren et al. 2001, Jiguet et al. 2007, Clavel et al. 2011). These individualistic responses could disrupt species interactions (Harrington et al. 1999, Schweiger et al. 2008), and lead to cumulative changes on community structure and composition (Warren et al. 2001, Menéndez et al. 2006, González-Megías et al. 2008, Devictor et al. 2012). As a result, ecological communities might become progressively dominated by generalist species (Warren et al. 2001, Menéndez et al. 2006, Wilson et al. 2007, González-Megías et al. 2008). This biotic homogenization of ecological communities (genetic, functional and taxonomic) could have a number of important implications for community and ecosystem properties and functioning, such as increased vulnerability to large-scale environmental events (Olden 2006, Clavel et al. 2011).

Bumblebees in mountain systems as a case study

Study species

Most of the examples on alterations in species distributions and/or abundances correlated with recent climatic trends correspond to species whose distributions are mostly limited by climate, or organisms that are highly mobile at some stage of their life cycle (e.g., Parmesan et al. 1999, Tingley et al. 2009). Such is the case of

flying insects, which are particularly sensitive to climatic conditions (Bale et al. 2002). Indeed, insects have responded to warming in all the predicted ways, from alterations in phenology and distribution (Parmesan et al. 1999, Diamond et al. 2011), to undergoing evolutionary adaptations (Thomas et al. 2001) and changes at the community level (González-Megías et al. 2008). Thus, there is ample evidence that insects are useful biological indicators to detect and evaluate environmental changes (Hughes 2000, Menéndez 2007).

Bumblebees (*Bombus* spp.) might be particularly well suited to test the impact of global change since they are cold-adapted insects mostly confined to temperate, alpine and arctic regions (Williams 1994, Fig. I.1). They are social insects that feed on pollen and nectar throughout their entire life (Fig. I.2); therefore, constant availability of pollen resources is necessary in order to complete the annual colony cycle. However, most bumblebee species have longer colony cycles than any flower resource, and most of them are generalists species, forced to switch to the most abundant flowers according to availability and profitability (Heinrich 1979). As a result, they are the most important pollinators at the special conditions to which they are adapted such as mountain ecosystems (Bingham and Orthner 1998), but also in temperate regions where they visit a wide variety of wild flowers and cultivated crops (Carvell et al. 2006, Rickets et al. 2008). Overall, their physiological characteristics and nutritional requirements could make bumblebees highly vulnerable to both climate warming, and changes in land use, respectively (Goulson et al. 2005, Williams et al. 2007, 2009).

Bumblebees are indeed suffering a worldwide decline, which has been associated to different components of the global change (Kosior et al. 2007, Grixti et al. 2009, Cameron et al. 2011). For instance, generalized alterations in bumblebee species distribution and abundance have been related to changes in land use and declines in preferred floral resources (Kosior et al. 2007, Kleijn and Raemakers 2008, Bommarco et al. 2011), and to infection by putatively introduced pathogens (Cameron et al. 2011). Bumblebee species differ markedly in their vulnerability to decline and it has been argued that these differences are related

both to a dietary (Goulson et al. 2005) and a climatic specialization (Williams et al. 2007, 2009).

Study system: The Cantabrian Range

Mountain areas occupy 24% of the Earth's land surface; they are hotspots of biodiversity and also harbour many ecological specialists and endemisms (Williams et al. 2003, Jetz et al. 2004, Körner and Ohsawa 2004). Montane fauna might respond more rapidly to climate change because the rate of warming is likely to accelerate in mountains (Nogués-Bravo et al. 2007). Although the heterogeneous topography in mountains can provide microclimate refugia to sustain local populations, habitable area for high-elevation species declines with increasing elevation (Wilson et al. 2005, Körner 2007). Hence, mountain systems worldwide are particularly fragile to changes in land use practices and changing climatic conditions (UNCED 1992, Körner and Ohsawa 2004, Schröter et al. 2005). Studies of species shifts in mountainous regions should therefore be a high priority in ecological and biogeographical studies in order to preserve mountain diversity.

Furthermore, mountain ecosystems are highly suitable to test organisms' responses to global change because they provide a changing distribution of species and climatic and environmental gradients over short spatial distances (Lomolino 2001, Körner 2007, Nogués-Bravo et al. 2007). Indeed, variability in temperature and precipitation patterns at regional scales, such as mountains, might be the most relevant way to evaluate ecological response to global change (Hughes 2000, Walther et al. 2002). For this reason, species' range shifts along mountain gradients can also reveal important patterns on community disassembly, which might be expected with climate change (Sheldon et al. 2011).

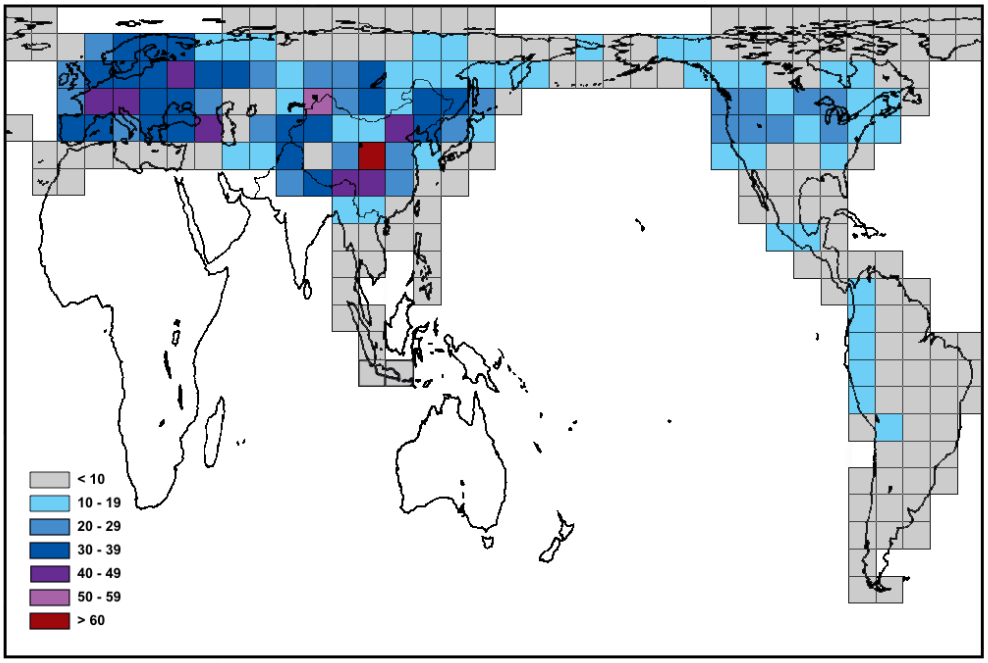

Figure I.1. Global distribution of bumblebee species richness, covering the known natural range of bumblebees. The colour scale indicates species richness. Grids are of equal area (about 611,000 km^2). Redrawn from Williams (1994).

Figure I.2. A worker of *B. terrestris*, a common and widespread species, pollinating an inflorescence of *Centaurea nigra*. Photograph by David Alvarez, with permission.

The Cantabrian Range (NW Spain) provides a strong elevation gradient near the sea, with a maximum elevation of 2650 m a.s.l. (Fig. I.3). The study area runs from 42.8–43.5° N to 4.5– 7.1° W. The climate of the region is Atlantic, with a mean annual temperature of 13°C and rainfall (*ca.* 1300 mm) distributed approximately evenly throughout the year. Dominant vegetation types at lower elevations include forest fragments standing out from a non-forest matrix mainly composed of pastures and heathlands in abandoned meadows, whereas shrubs and subalpine pastures dominate the vegetation above 1600 m a.s.l. (García et al. 2005, Fig. I.3).

The Cantabrian Range has an exceptionally high diversity of bumblebee species, with up to 14 species occurring in individual sites. This is largely attributable to the high density and diversity of forage provided by heathlands and pastures, and an extensive farming system, with small fields, which is still in use (Fig. I.3). Overall, 24 bumblebee species have been described in the region, excluding parasitic bumblebees belonging to the sub-genus *Psithyrus* (Obeso 1992). The regional fauna includes rare species such as *B. gerstaeckeri* or *B. inexpectatus* (Rasmont and Iserbyt 2010). Moreover, the study region constitutes the southern limit of distribution for many widespread European bumblebees, such as *B. soroeensis* and *B. wurflenii* (Rasmont and Iserbyt 2010).

Following the global trend in climate warming, climate data from meteorological stations show that temperature in the region has risen by *ca.* 0.8°C between 1971 and 2000 (Fig. I.4), whilst no changes in precipitation patterns have been found (IPCC 2007, Álvarez et al. 2009). While it is true that this increase in temperature is slightly less than the 0.9°C for the northern hemisphere as a whole during the same period (IPCC 2007), the time span considered by this study, from 1988-1989 to 2007-2009, is extremely likely to be representative of current climate warming. For this reasons, the Cantabrian Range represents an ideal mountain system for studying the effects of climate change on bumblebee assemblages over recent decades.

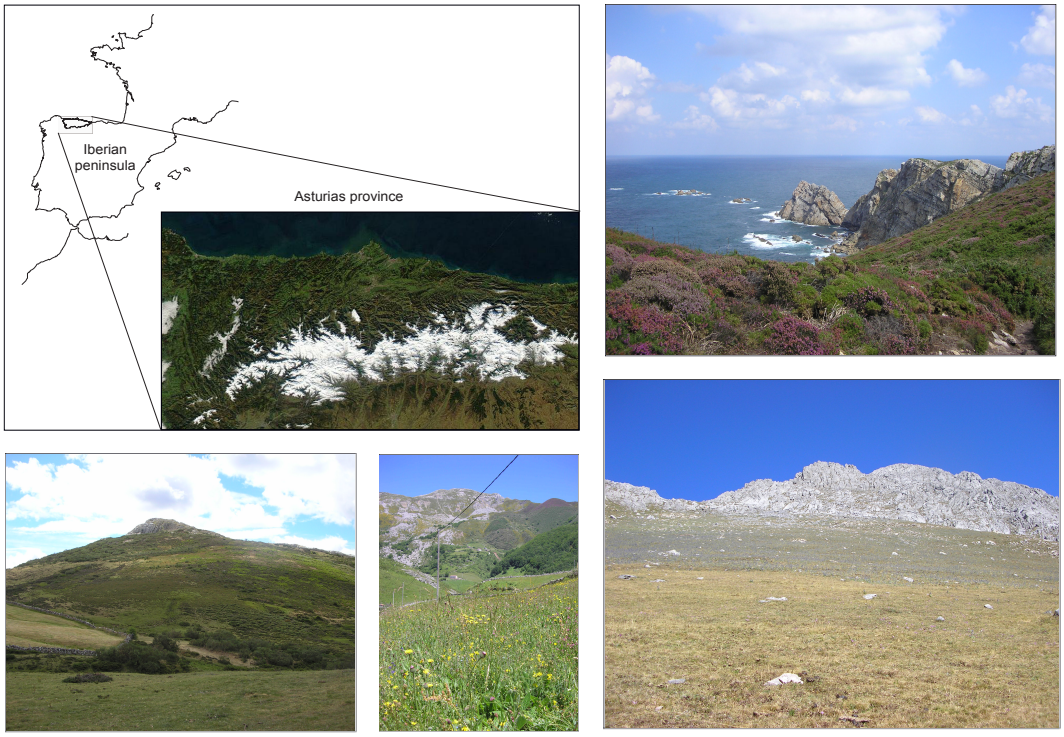

Figure I.3. Satellite image of the Cantabrian Range (The Visible Earth, NASA, http://visibleearth.nasa.gov/) and some of the sampling sites, dominated by pastures and shrublands (Photographs by Emilie F. Ploquin).

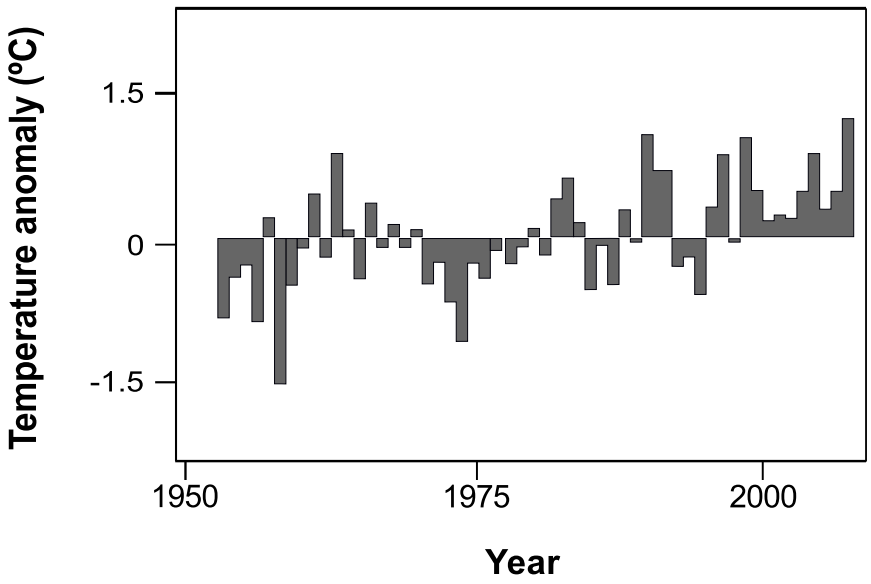

Figure I.4. Temperature anomalies (means) in the study region between 1950 and 2006 relative to the mean for 1960-90. Redrawn from Álvarez et al. 2009.

Specific aims and structure of the thesis

This thesis has focused on the potential changes in distribution and abundance of bumblebee species in a mountain range where many of the species have their southern limit of distribution. To carry out this study, we re-surveyed data regarding bumblebee distribution along almost the complete elevation range present in the study region (0-2200 m a.s.l.). In addition, we aimed to ascertain whether these changes could be explained by species-specific traits and evaluate the potential effect of climate change and land cover on the changes observed.

The specific aims of this thesis are developed in four chapters (Fig. I.5).

Chapter 1. The main objective is to investigate changes in abundance and distribution of bumblebee species over the last two decades in mountain areas of the Cantabrian Range. To achieve this, we re-surveyed bumblebee assemblages on the Cantabrian Range in 2007-2009, 20 years after the previous survey in 1988-1989 (Fig. I.5). Changes in species' upper and lower boundaries, mean elevation and elevation extents were assessed. Recent and historical bumblebee assemblages located at lower, medium and upper elevations were compared to determine whether changes in elevation range have entailed concomitant alterations in patterns of community composition. Species turnover was associated with colonization and extinction events and also with variability in the abundance of the species. Extinction events were especially relevant at medium elevations, while only upper communities had a positive net outcome between colonization and extinction events. The combination of these effects resulted in the homogenization of bumblebee assemblages, especially between medium and upper elevations.

Chapter 2. The main goal of this study is to investigate the role of ecological and life history traits in determining species-specific responses to climate warming over two decades in montane areas of the Cantabrian Range (Chapter 1, Fig. I.5). First, we estimated the relative change in the local climatic conditions at which each species occurred between their historical (1988-1989) and recent (2007-

2009) distribution. Then, we examined the relationship between species' traits and changes in climate space. We found that, irrespective of phylogenetic constraints, climatic tolerance, though not other ecological traits, strongly determined each individual species' response. Specifically, bumblebee species with broad climatic tolerance showed a reduction in their elevational range and greater changes in the climatic conditions at which they occurred from one period to another.

Chapter 3. The aim of this chapter is to evaluate the role of both climatic and land-cover variables on bumblebee species distribution and abundance in the study region (Fig. I.5). These factors were analyzed at increasingly nested neighbourhood distances (100-3000 m) as different bumblebee species may respond at different spatial scales. We also used the historical dataset from 1988-1989 to calibrate the current distribution and abundance models and to test predictions on the relative change occurring over the last two decades. Specifically, we addressed whether current estimated distribution and abundance were congruent with historical bumblebee distribution patterns and evaluated the role of climate and landscape context at explaining potential changes in species distribution and abundance between both study periods. Bumblebee presence and abundance patterns in the Cantabrian Range were found to be mainly determined by climatic variables (notably temperature), rather that land-cover variables.

Chapter 4. In this chapter we compare the trophic niche width and niche overlap in bumblebee assemblages with different species richness across the elevational gradient. Bumblebee assemblages were selected based on the 2007-2009 survey (Fig. I.5). We calculated the trophic niche width and niche overlap between coexisting species by means of stable isotope analysis. Stable isotope analysis is an indirect approach that provides a time- and space-integrated record of resource assimilation because the stable isotopes in the tissue of consumers reflect the stable isotope signature of the diet (Fry et al. 1978, Dalerum and Angerbjörn 2005). Bumblebee species showed narrower trophic niches and a greater degree of niche overlap between species pairs in species-rich assemblages, suggesting a more specialized diet and stronger interspecific competition in those communities.

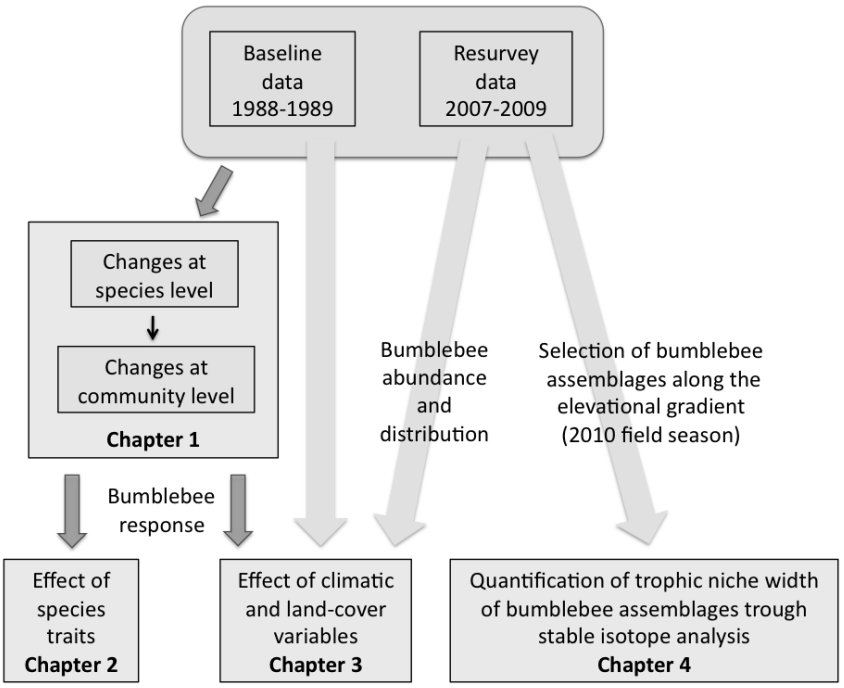

Figure I.5. Diagram showing the objectives that are studied throughout this thesis and summary of field surveys according to their contributions to each chapter. Darker arrows indicate that both databases were used for that specific objective, while pale arrows indicate that only one of the databases was used.

The results presented herein provide an increase in knowledge of the response to environmental change of bumblebees in mountain systems. Besides, we provide evidence of the suitability of bumblebee species in mountain systems as an ecological framework for testing the impact of global change. The patterns and processes described have applied implications for conservation, as they allow the prediction of the vulnerability of bumblebee species given their susceptibility in the face of global change.

1

Bumblebee community homogenization after uphill shifts in montane areas of northern Spain

INTRODUCTION

Widespread alterations in the patterns of species distribution and abundance reflect the ecological impact of global environmental change on biodiversity (Parmesan and Yohe 2003, Hickling et al. 2006, Lenoir et al. 2008, Chen et al. 2011*b*). These alterations include extinction from historical sites and colonization of new ones in response to the displacement of suitable ecological conditions (e.g., climate, habitat and food-resource availability) to which species are adapted, resulting in range shifts (Parmesan et al. 1999, Parmesan 2006). Because organisms perceive and respond to the environment in a species-specific way, cumulative changes in patterns of community composition are also expected (Parmesan 2006, Wilson et al. 2007, Devictor et al. 2012). Indeed, certain patterns of extinction and colonization events could even result in a biotic homogenization between biological communities rather than in a higher differentiation between them (McKinney and Lockwood 1999, Olden 2006). However, while alterations in both the distribution and abundance of individual species have been widely documented (e.g., Parmesan et al. 1999, Parmesan and Yohe 2003, Wilson et al. 2005, Hickling et al. 2006, Lenoir et al. 2008), studies at the community-level are still scarce and therefore empirical evidence testing the above hypotheses remain largely elusive (Olden 2006).

Far from being trivial, filling this gap in our knowledge may have important ecological, evolutionary and conservation implications. This is especially true for those species whose population dynamics as well as community structure strongly influence key ecological processes as well as ecosystem services that benefit society such as pollination (Hegland et al. 2009, Potts et al. 2010). This is the case of bumblebee species (*Bombus* spp.), which are cool adapted, endothermic species and thus well adapted for activity in cool conditions where most pollinators are inactive (Heinrich 1979). As a result, they are the most important pollinators at the special conditions to which they are adapted such as mountain ecosystems (Bingham and Orthner 1998), but also in temperate regions (Ricketts et al. 2008).

Furthermore, all bumblebees have similar ecology but for their tongue length, which allows them to visit a wide variety of wild flowers and cultivated crops (Carvell et al. 2006, Ricketts et al. 2008).

Like many other pollinator species, bumblebees are not unaware to global change and they are experiencing an ongoing worldwide decline (Kosior et al. 2007, Grixti et al. 2009). Indeed, generalized alterations of bumblebee species distribution and abundance are widely recognized in regions as diverse as Western and Central Europe (Kosior et al. 2007, Williams et al. 2009, Bommarco et al. 2011), Asia (Williams et al. 2009) and North America (Grixti et al. 2009, Cameron et al. 2011). Yet, the currently available data on bumblebee decline is strongly biased toward studies investigating geographical (i.e., latitudinal) extinctions of species over time, while studies explicitly evaluating patterns of change of bumblebee species from a community-level approach are still surprisingly scarce in scientific literature (but see Bommarco et al. 2011, Dupont et al. 2011). On the other hand, potential shifts towards higher elevations in mountain areas have been neglected, although such effects are particularly expected to occur given the thermal sensitivity of bumblebee species (Heinrich 1979).

In the present study, we sought to investigate elevational shifts in bumblebee species over the last two decades in mountain areas of the Cantabrian Range (NW Spain) and determine whether these changes have entailed concomitant alterations on patterns of community composition. Mountain ecosystems are highly suitable for this purpose because they provide climatic and environmental gradients over short spatial distances (Lomolino 2001, Körner 2007, Nogués-Bravo et al. 2008). The Cantabrian Range is an ecotonal zone between the Eurosiberian and the Mediterranean regions in Europe, thus harbouring bumblebee species from both boreal and Mediterranean fauna (Rasmont and Iserbyt 2010). Overall, 24 bumblebee species have been described in the region (Obeso 1992), including rare species such as *Bombus gerstaeckeri* or *B. inexpectatus*. Recent evidence suggests that climatic conditions in the region are changing as climate data from meteorological stations shows that temperature in the region has risen by *ca.* 0.8°C since 1970 to date (IPCC 2007, Álvarez et al.

2009). We used historical (1988-1989) and recently re-surveyed (2007-2009) data regarding bumblebee distribution along almost the complete elevational range present in our study region (0-2200 m a.s.l.). Given the persistent rise in global temperatures and the thermal sensitivity of bumblebee species (see references above) we predict a generalized migration of individual species towards upper elevations. Furthermore, due to potential differences in species's abilities to shift ranges in response to climate change, alterations of patterns of community structure are also expected. Because rising temperatures are showed to become particularly relevant as elevation increases (Körner 2007, La Sorte and Jetz 2010), we expect that these effects (both at species and community level) will be stronger on bumblebee assemblages located at intermediate and high elevations.

MATERIAL AND METHODS

Study sites and sampling design

The study was carried out mostly in the Asturias region of the Cantabrian Range (NW Spain; Fig 1.1). The study area approximately extends from 42.8–43.5° N to 4.5– 7.1° W. The climate of the region is Atlantic, with a mean annual temperature of 13°C and rainfall (*ca.* 1300 mm) distributed approximately evenly throughout the year. Sampling localities varied from sites located at sea level, to those at the top of mountains in the Cantabrian Range at approximately 2200 m a.s.l., thus comprising an elevational range sufficiently representative of almost all climatic conditions and suitable habitats for bumblebees in our study region (maximum elevation 2650 m a.s.l). This region presents a high diversity of bumblebee species (a total of 24 species excluding species of sub-genus *Psithyrus*; Obeso 1992) and constitutes the south-eastern distribution limit for many of them (Rasmont and Iserbyt 2010). The vegetation in this region is dominated by mowed meadows near the coast. Deciduous mature forests dominate between 700 and 1600 m a.s.l. (montane lower limit and tree line, respectively, Díaz and Fernández 1987). Due to

traditional cattle ranging and selective logging, however, the current regional landscape contains remnant forest fragments standing out from a non-forest matrix mainly composed of pastures, heathlands in abandoned meadows and areas of shallow soil, and scattered small villages. At elevations over 1600 m, shrubs and subalpine meadows dominate the vegetation up to at least 2500 m. Overall sites differed in the dominant habitats present in the area, but sites located at the same elevations shared climatic conditions and land-use patterns (García et al. 2005, *pers. obs.*).

We sampled bumblebee assemblages in 1988-1989 (Obeso 1992) and 2007-2009 in open habitats (meadows, heathlands and alpine pastures) stratified by elevation. We included data from 82 sites that were visited once in 1988-1989, and from 54 sites during 2007-2009 (Fig 1.1). In 1988-1989, a sample of individuals of all species that were observed during each visit was recorded, but each site was visited only once. Thus, in order to standardize sample effort along the elevational gradient between both study periods, we repeated carefully the same procedure and number of visits per site as reported in Obeso (1992). However, some of the original sites were no longer suitable for sampling, so we used a random selection procedure as it is the most appropriate to detect trends in community changes of species at a regional scales, as long as the sites selected are equivalent and sampling effort is the same (Shaffer et al. 1998). We performed individual-based rarefaction analysis to samples along the elevational gradient to allow comparisons of species richness between two periods with different sampling sizes (Gotelli and Colwell 2001; see Appendix 1.1).

We sampled each site throughout the spring and summer seasons (mid May to late August) of 2007-2009. At each site, we conducted time-constrained transects throughout a circular area of 100 m radius. Each locality was visited once and sampling was continued until 30 min had been spent at that site by two collectors (or 1 h by one collector). All searches were conducted between 09.00 and 17.00 h and during warm, dry weather favourable to bee activity. Surveys were carried out during maximum flower blooming in order to avoid differences in the composition of bumblebee assemblage between localities due to variations in

resource availability. Foraging individuals were caught with a hand net and most of them were collected for identification in the laboratory. We identified all bumblebees to the species level and determined their caste and abundance.

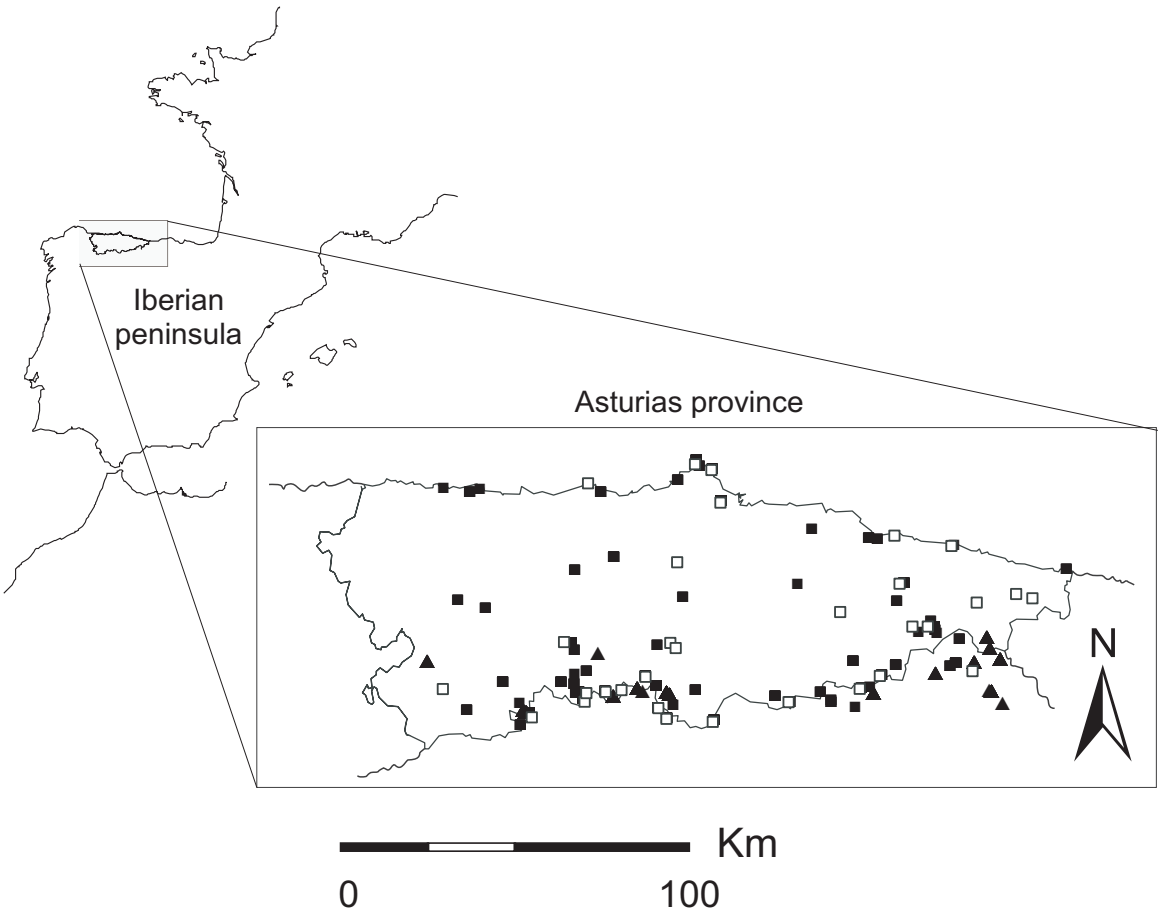

Figure 1. Location of sample sites in the Asturian Region of the Cantabrian Range (NW Spain). Filled squares indicate sites sampled in 1988-1989 (*n* = 49) and triangles refer to sites sampled in 2007-2009 (*n* = 21). Open squares indicate sites sampled in both 1988-1989 and 2007-2009 (*n* = 33).

Spatial constraints

Spatial autocorrelation is an important issue when analysing geographical patterns of species diversity (Legendre and Legendre 1998). Spatial autocorrelation in explanatory (i.e., elevation) and response (i.e., species presence, abundance and assemblage) variables was therefore investigated prior to the analysis with Moran's I coefficient. Moran's I calculates the similarity between observations from pairs of locations, for each distance class and for each explanatory variable. The number of equidistant distance classes was calculated following Sturge's rule ($N_{\text{classes}} = 1 + 3.3 \log n$). Because autocorrelation was found in some response variables (abundance of some particular species) we applied a trend surface analysis (TSA) (Legendre and Legendre 1998) to each of these variables to remove the spatial trend (detrending was corroborated by Moran's I correlograms on TSA residuals, data not shown). Thus, subsequent analysis involving the abundance of these species as a response variable was performed with these spatially independent residuals. We also tested for spatial autocorrelation in the spatial distribution of sampling locations across elevations between both study periods. Moran's I correlograms did no produce significant coefficients at any distance class ($P > 0.05$ in all pair comparisons; data not shown), thus ensuring that all localities were homogeneously distributed and that there was no spatial aggregation among elevations for any of the sampling periods (Legendre and Legendre 1998). Accordingly, we ensured a suitable spatial framework to test temporal changes in bumblebee assemblages irrespective of spatial constraints in sampled sites.

Calculations and statistical analysis

We divided our analysis into two main steps: a species and a community-level approach. To examine changes at the species level, we first calculated mean elevation, median and both maximum and minimum elevational limit in both periods with the aim to investigate changes in the maximum and minimum elevational limit at which each individual species occurred in each period, as well as variations in the elevational range (i.e., the difference between the maximum

and minimum elevational limit in the second period relative to that in the first period). Because of inevitably differences in catch sizes between periods, and in order to obtain statistically-tested differences in species distribution between both study periods, these measures were extracted by using a highly conservative approach in order to ensure that our conclusions are robust. Thus, we randomly subsampled the data so that the same number of individuals was "sampled" in each period (*ca.* 60% of the total number of individuals for each species; Table 1.1), with exactly the same elevational distribution of catch in both periods (see Chen et al. 2011*a* for a similar procedure). The subsampling was repeated 500 times per species in order to obtain means (and deviations) of change in the average mean elevation, upper/lower boundaries, as well as the variations in elevational ranges for each bumblebee species. Differences between periods in these measures were tested by one-sample *t*-test (H_0: $\mu = 0$ m; see Appendix 1.2).

We also investigated whether changes in the distribution of individual species entailed concomitant changes in community sorting. To compare the assemblage of bumblebee species across elevations, we used detrended correspondence analysis (DCA), a multivariate technique that orders plots based on the number of individuals of each species (Hill and Gauch 1980). DCA is especially suitable for our purpose given that they take under consideration the tendency for one-dimensional gradients to be distorted into an arch such as those produced by elevation (Hill and Gauch 1980). DCA separates sources of variation into different axes, allowing the examination of different factors on overall community composition. The factors explaining the greatest amount of variation in the data separate plots along the primary DCA axis (hereafter DCA1), while factors explaining a lesser amount of variation separate plots along the secondary axis of variation (DCA2). We downweighed rare species, and the number of segments considered for rescaling axes was 26 (Hill and Gauch 1980). The interpretation is relatively simple: ordination scores closer to one another in ordination space are more similar in species composition than those that are further apart. Our ordination used the total number of each bumblebee species recorded at each study site. In order to simplify the interpretation, we divided the elevational

gradient into sites located at lower (0 – 900 m), medium (900 – 1500 m) and upper (> 1500 m) elevations. We used this arbitrary classification since these ranges were strongly related to vegetation thresholds in our study region and overlapped with the mean elevational displacement of the different species (see Results). We used ANOVA to test the differences in bumblebee assemblages between these elevational ranges.

To investigate extinction and colonization events at different elevations, we divided all sampled sites into 100 m intervals in order to increase the number of available comparisons between study periods. We therefore grouped sites within a total of 23 100 m elevational intervals given that localities varied from 0 to 2218 m (see Wilson et al. 2005 for a similar procedure). We considered a colonization event to be when a species was not recorded at a given elevation during the first period but was present in the second, and vice versa for an extinction event (see González-Megías et al. 2008 for a similar approach). We calculated the number of species that colonized and became extinct between the two study periods (and their abundance) at lower, medium and upper elevations. All statistical analyses were performed in R statistical environment (R Development Core Team 2012). To develop DCA's we used the decorana function from the "vegan" statistics package (Oksanen et al. 2011) and the "BiodiversityR" package was used to generate rarefaction curves (Kindt and Coe 2005).

RESULTS

We caught 1692 specimens of bumblebees in the second (2007-2009) period, representing 21 species, against the 1254 from 24 species that were caught in the first (1988-1989) period (Table 1.1). All bumblebee species sampled in the first period were also caught in the second one except *B. laesus*, *B. gestaeckeri* and *B. cullumanus*. In addition, some species such as *B. mucidus, B. ruderatus, B. hypnorum, B. monticola, B. subterraneus* and *B. inexpectatus* were seldom caught in 2007-2009 as they already were in 1988-1989 (Table 1.1).

Changes at the species level

A total of 11 species increased their abundance in the second relative to the first period, while 10 species decreased their abundance from the first to the second period. However, these differences were only significant for *B. lucorum* (ANOVA; $F_{1,62}$ = 4.84, P < 0.05; for all the remaining species P > 0.05; data not shown). Rarefaction curves suggest that sampling effort for characterising bumblebee fauna in the Cantabrian Range was adequate as species richness reached an asymptote in both study periods (see Appendix 1.1).

There were significant differences in species distribution across elevations between the two study periods (Table 1.1, Fig. 1.2; see Appendix 1.2). More than half of the species considered increased their mean elevation (n = 9; 56.2%) while the opposite was true for 7 species (43.7%). Changes in mean elevation across species in 2007-2009 relative to 1988-1989 varied from a decrease of 531 m in *B. hortorum* to an increase of 717.8 m in *B. muscorum*. Most of the species retracted their lower boundary (n = 11, 68.7%), while 8 species (50.0%) expanded their upper boundary. Besides, 6 species (37.5%) retreated at both their lower and upper boundaries. Overall, these shifts on lower and upper boundaries resulted on substantial changes in the elevational range across species between periods. While 11 species (68.7%) narrowed their elevational range in 2007-2009 relative to 1988-1989 (ranging from a decrease of 16.9 m in *B. hortorum* to 1229.6 m in *B. wurflenii*), only three species increased their elevational range (ranging from an increase of 200.9 m in *B. muscorum* to 821.3 m in *B. jonellus*; Table 1.1; Fig. 1.2). Changes in elevational range were significantly related to variations in their upper elevational limit ($F_{1,14}$ = 8.74, R^2 = 0.38, P < 0.05) but especially to alterations in the lower elevational limit of species distribution (linear regression; $F_{1,14}$ = 71.55, R^2 = 0.84, P < 0.0001). This means that the observed reductions in the elevational range was especially related to increases in the lower limit of species (that is, increases in the lower elevational limit at which species occurred) rather than decreases in their upper elevational limit (Fig. 1.2).

Table 1.1. Tongue length attributes of bumblebee species and elevational shifts (in metres) between 1988-1989 and 2007-2009 (mean ± SE).

Species	Tongue length†	NoI	NoL	Mean elevation	Lower boundary	Upper boundary	Elevational extents
B. cullumanus ‡	Short	(18, 0)	(9, 0)	---	---	---	---
B. gerstaeckeri ‡	Long	(2, 0)	(1, 0)	---	---	---	---
B. hortorum	Long	(142, 74)	(38, 17)	-522.7 ± 134.8 ***	-8.0 ± 31.8 ***	-7.1 ± 159.0	-16.8 ± 193.9 *
B. humilis	Medium	(11, 129)	(6, 21)	-263.1 ± 74.1 ***	-110.5 ± 85.6 ***	574.5 ± 299.5 ***	721.2 ± 270.0 ***
B. hypnorum ‡	Short	(6, 2)	(3, 1)	---	---	---	---
B. inexpectatus ‡	Medium	(4, 6)	(3, 1)	---	---	---	---
B. jonellus	Short	(30, 47)	(11, 12)	88.3 ± 97.6 ***	-615.3 ± 186.2 ***	203.9 ± 19.8 ***	821.3 ± 170.2 ***
B. laesus ‡	Short	(9, 0)	(4, 0)	---	---	---	---
B. lapidarius	Short	(86, 154)	(16, 22)	291.5 ± 100.6 ***	570.2 ± 421.4 ***	61.7 ± 271.8 ***	-529.1 ± 470.2 ***
B. lucorum	Short	(92, 214)	(30, 34)	395.3 ± 120.1 ***	252.2 ± 389.4 ***	273.4 ± 213.6 ***	-3.0 ± 471.5
B. mesomelas	Medium	(98, 81)	(26, 15)	67.1 ± 93.9 ***	642.9 ± 177.3 ***	-1.0 ± 14.4	-626.0 ± 172.7 ***
B. monticola	Medium	(11, 15)	(4, 6)	-230.0 ± 59.7 ***	178.6 ± 63.3 ***	-32.5 ± 89.28 ***	-206.9 ± 105.2 ***
B. mucidus ‡	Short	(4, 20)	(3, 6)	---	---	---	---
B. muscorum	Medium	(65, 22)	(30, 10)	717.8 ± 121.4 ***	61.5 ± 53.8 ***	258.1 ± 148.5 ***	200.9 ± 157.2 ***
B. pascuorum	Long	(116, 16)	(33, 7)	-19.7 ± 95.0 ***	-16.9 ± 23.9 ***	210.7 ± 271.6 ***	-240.6 ± 280.9 ***
B. pratorum	Short	(55, 19)	(28, 14)	396.6 ± 159.9 ***	42.5 ± 67.5 ***	216.6 ± 99.8 ***	-166.5 ± 136.0 ***
B. ruderarius	Medium	(34, 103)	(14, 19)	108.0 ± 74.8 ***	429.0 ± 553.7 ***	22.9 ± 179.7 **	-387.6 ± 574.8 ***
B. ruderatus ‡	Long	(17, 4)	(7, 3)	---	---	---	---
B. sichelii	Short	(56, 118)	(12, 21)	-145.4 ± 107.6 ***	-34.3 ± 132.3 ***	-75.3 ± 121.5 ***	-39.9 ± 180.0 ***
B. soroeensis	Short	(102, 327)	(19, 27)	-99.8 ± 124.6 ***	226.7 ± 166.1 ***	-116.2 ± 233.0 ***	-321.7 ± 298.1 ***
B. subterraneus ‡	Long	(5, 3)	(2, 3)	---	---	---	---
B. sylvarum	Medium	(46, 19)	(16, 9)	109.9 ± 132.1 ***	3.4 ± 3.5 ***	-51.7 ± 272.8 ***	-22.6 ± 318.6
B. terrestris	Medium	(189, 278)	(35, 29)	-73.6 ± 167.4 ***	9.1 ± 10.2 ***	-1.4 ± 280.8	2.3 ± 294.1
B. wurflenii	Short	(56, 41)	(18, 10)	315.1 ± 66.1 ***	1151.5 ± 245.8 ***	-69.7 ± 149.1 ***	-1229.6 ± 298.6 ***

NoI, NoL: number of individuals and localities in the first and second period, respectively. ‡ *t*-tests were not performed because number of individuals was below ten in at least one period. Significance levels are based on one-sample *t*-tests: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

† Short: ≤8 mm; medium: 8-9 mm; long: >9 mm. Data derived primarily from Obeso (1992).

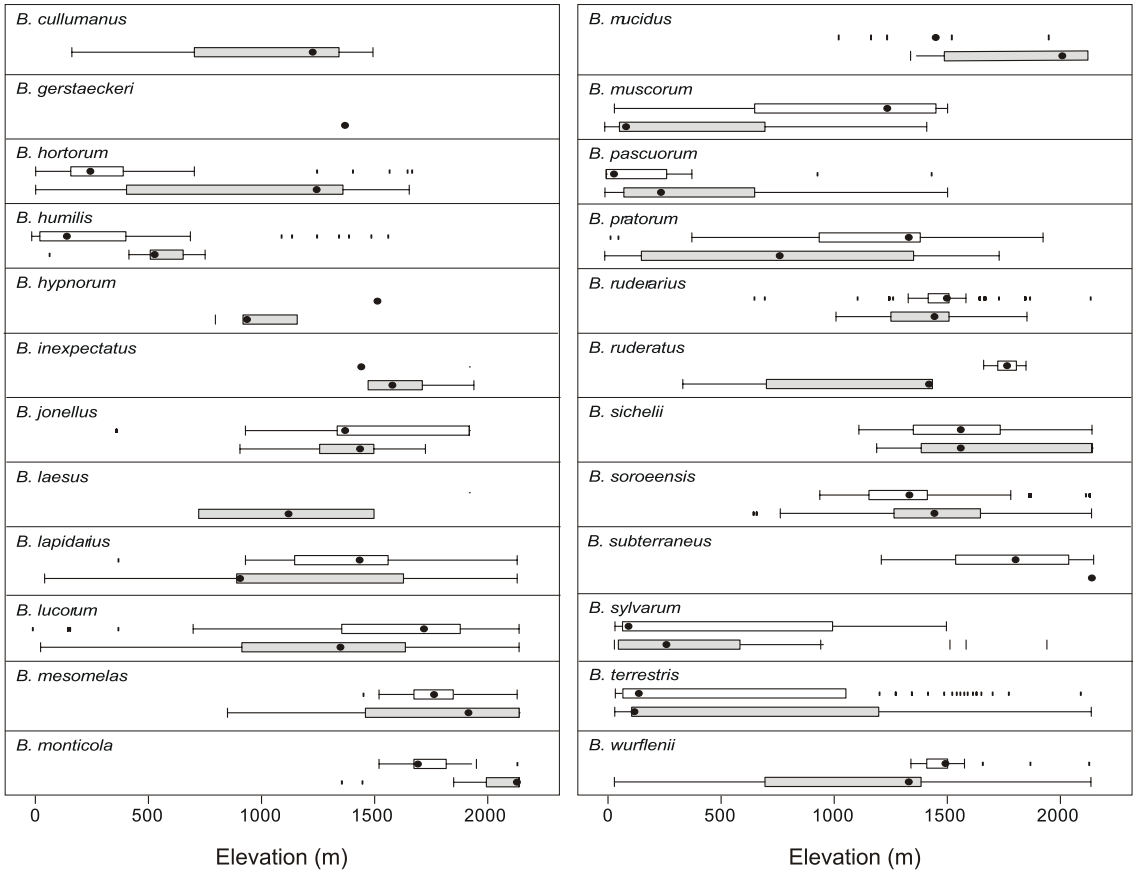

Figure 1.2. Boxplot of elevational distribution of bumblebee species (*Bombus* spp.) in the 1988-1989 (grey boxes) and 2007-2009 (white boxes) periods. Boxes indicate the interquartile range of the data and the position of the median (dot), whisker length = 1.5 SD, outliers are plotted individually.

Changes at the community level

Elevation was highly significantly related to the primary DCA axis (i.e., DCA1) in both 1988-1989 ($F_{1,79}$ = 167.9, R^2 = 0.68, P < 0.0001) and 2007-2009 ($F_{1,52}$ = 130.7, R^2 = 0.71, P < 0.0001), but not along the DCA2 in either of the two study periods (all P > 0.05). This suggests that much of the variation in bumblebee assemblages in each period was due to differences in the elevation at which species occurred in the different sampling periods. In 1988-1989, there were significant differences in DCA ordination scores between elevations suggesting a clear distinction in species

assemblage occurring at lower, medium and upper elevations (Fig. 1.3 and 1.4a; all pair-wise comparisons using Tukey-Kramer HSD were significant at $P < 0.05$). However, in 2007-2009 there were no significant differences between bumblebee assemblages located at medium and upper elevations ($P > 0.05$; Fig. 1.4b). Furthermore, in the 2007-2009 period the differences between assemblages located at lower and upper elevations increased relative to the 1988-1989 period (Fig. 1.4). Generally, bumblebee assemblages at present had much lower abundances of long-tongued species (Fig. 1.5). In addition, short-tongued bumblebees become dominant species at medium and upper assemblages from one period to another (Fig. 1.5).

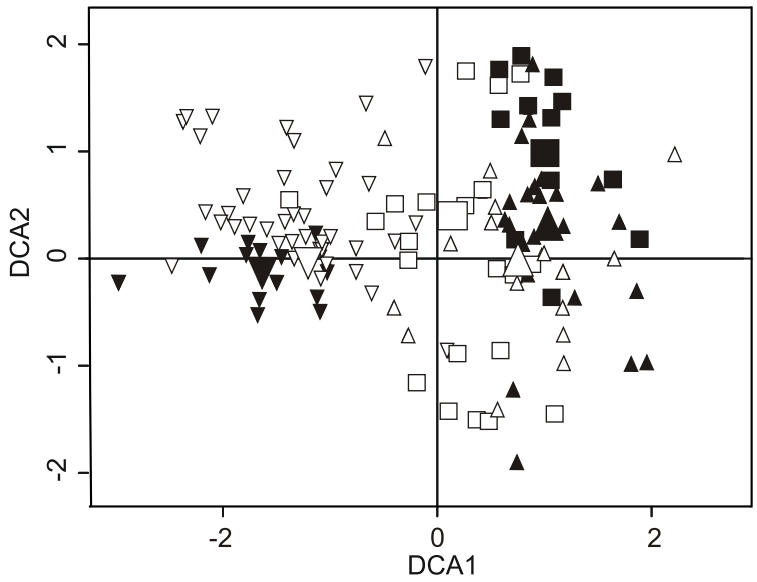

Figure 1.3. Detrended correspondence analysis (DCA) of bumblebee community composition in the Cantabrian Range (NW Spain). Symbols represent the species assemblage at each of the 135 sampled sites in the 1988-1989 (white symbols) and 2007-2009 (black symbols) sampling periods. The different shapes correspond to species assemblage at lower (inverted triangles, 0 – 900 m a.s.l.), medium (quadrats, 900 – 1500 m) and upper (triangles, > 1500 m) elevations. Larger symbols refer to the mean ordination scores for each elevation.

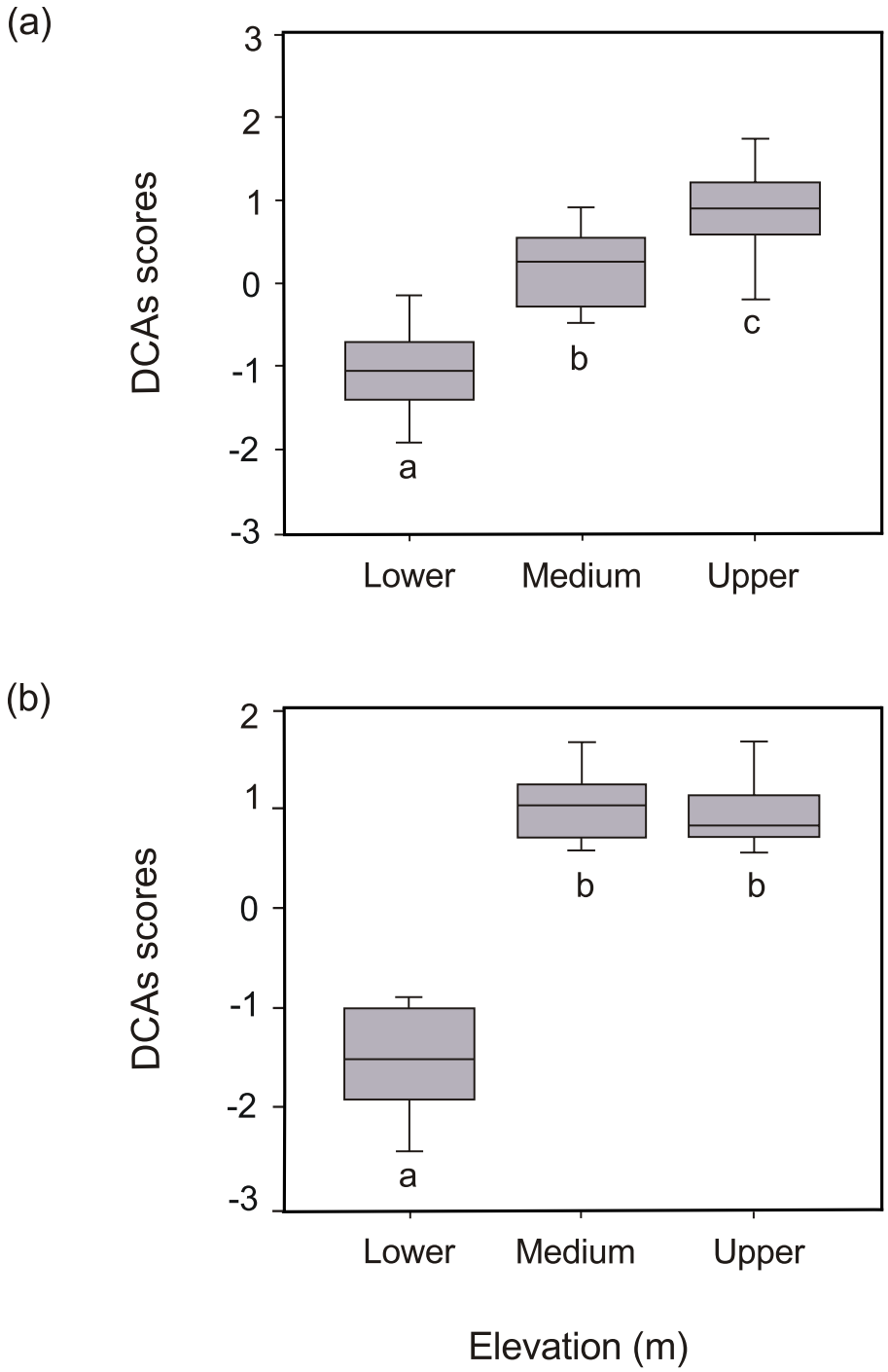

Figure 1.4. Boxplot comparing the DCA scores for sampled sites at lower, medium and upper elevations in 1988-1989 (a) and 2007-2009 (b). Boxes with the same letter do not differ significantly.

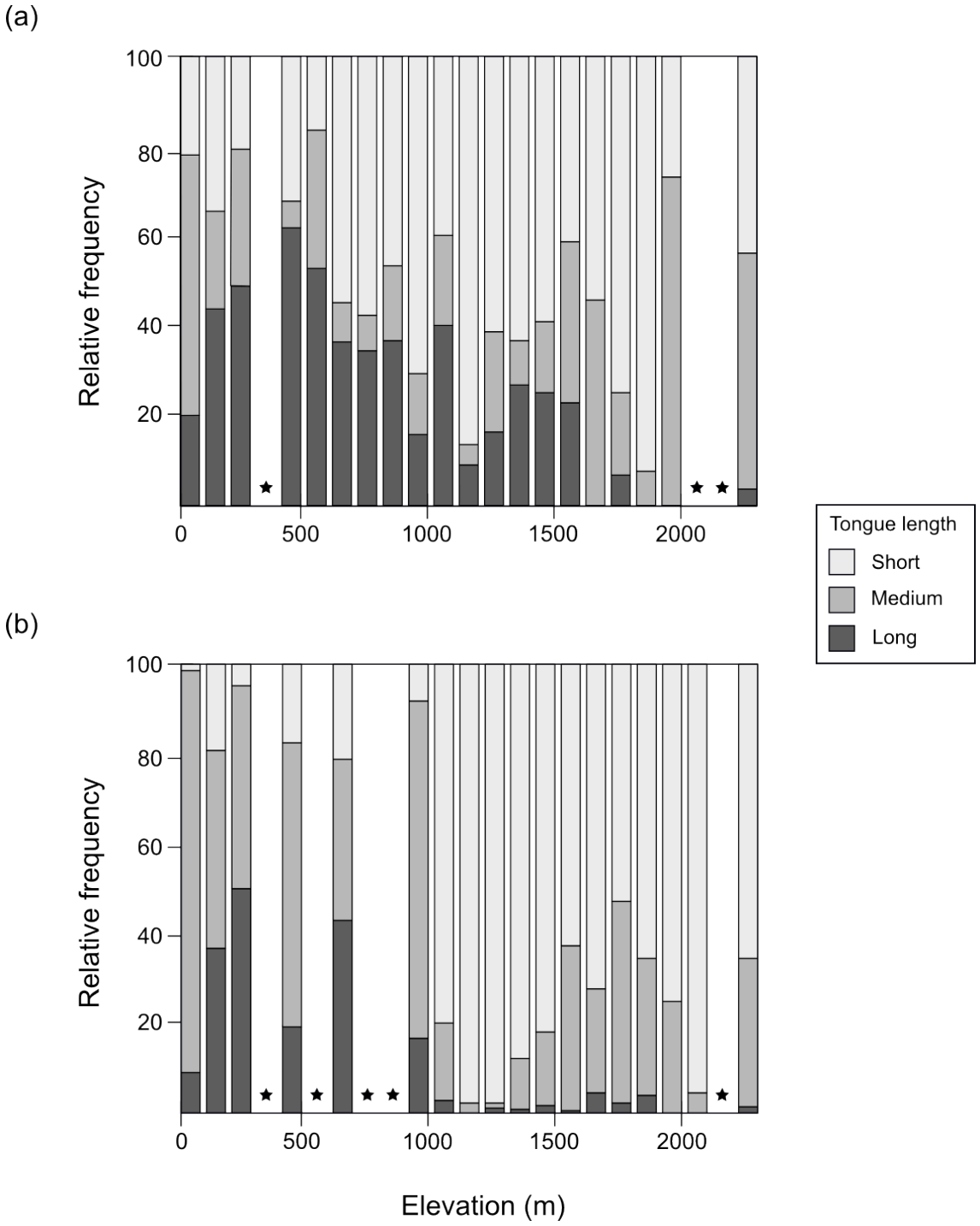

Figure 1.5. Relative abundance of bumblebees grouped by their tongue length across elevations in the 1988-1989 (a) and 2007-2009 (b) periods. Values are standardized to 100% to homogenize. Asterisks represent altitudinal ranges in which there were no sampling sites (see text for details).

Colonization and extinction events

The maximum number of species colonizing a site between 1988-1989 and 2007-2009 ranged from three species at lower elevations to eight species at upper elevations (Table 1.2). These new species formed on average 27.1%, 25.2% and 34.7% of the total number of species present at lower, medium and upper elevations, respectively (Table 1.2). Indeed, 8.5%, 9.1% and 17.7% of species abundance found at lower, medium and upper bumblebee assemblages during the second period were in fact colonizing species.

The number of species becoming extinct between 1988-1989 and 2007-2009 ranged from six species at both upper and lower elevations to ten species at medium elevations (Table 1.2). This accounted for nearly 50% of species relative to the first sampling at both lower and medium elevations, but only 25.7% of species from communities at upper elevations (Table 1.2). Extinctions exceeded colonizations at both lower and medium elevations with only upper elevations having a positive outcome between colonization and extinction events (Fig. 1.6). The net loss of species (resulting from the difference between colonization and extinction events) was higher at medium (3.4 ± 3.0) than at lower elevations (2.6 ± 0.1; Fig. 1.6).

Table 1.2. Summary of the number of species at each elevational range in the first (1988-1989) and second (2007-2009) periods, the number of species present only in the second period (colonizing species) and the number of species present in the first period that were extinct by the second (extinct species). Percentages (%) of species in the 2007-2009 assemblages that had colonized between the first and second period, species present in both periods (established species) and present in the first period that were extinct by the second (extinct), and colonizing species abundance are also shown. Mean ± SE (range).

	Total number of species		**Colonizing species**	**Extinct species**	**Colonizing species (%)**	**Established species (%)**	**Extinct species (%)**	**Abundance of colonizing species (%)**
	1st	**2nd**						
Lower	8.7 ± 1.6 (7, 11)	6.0 ± 1.8 (3, 8)	1.8 ± 1.4 (0, 3)	4.4 ± 1.8 (2, 6)	27.1 ± 26.3	66.5 ± 22.7	51.6 ± 21.6	8.5 ± 1.7
Medium	12.1 ± 4.9 (11, 17)	10.2 ± 5.0 (6, 17)	2.2 ± 2.0 (0, 4)	6.2 ± 2.3 (4, 10)	25.2 ± 20.3	74.4 ± 20.5	49.1 ± 20.2	9.1 ± 4.8
Upper	10.5 ± 4.5 (5, 18)	11.0 ± 2.3 (7, 14)	3.8 ± 2.4 (0, 8)	2.5 ± 1.8 (1, 6)	34.7 ± 25.5	65.6 ± 25.8	25.7 ± 18.5	17.7 ± 8.5

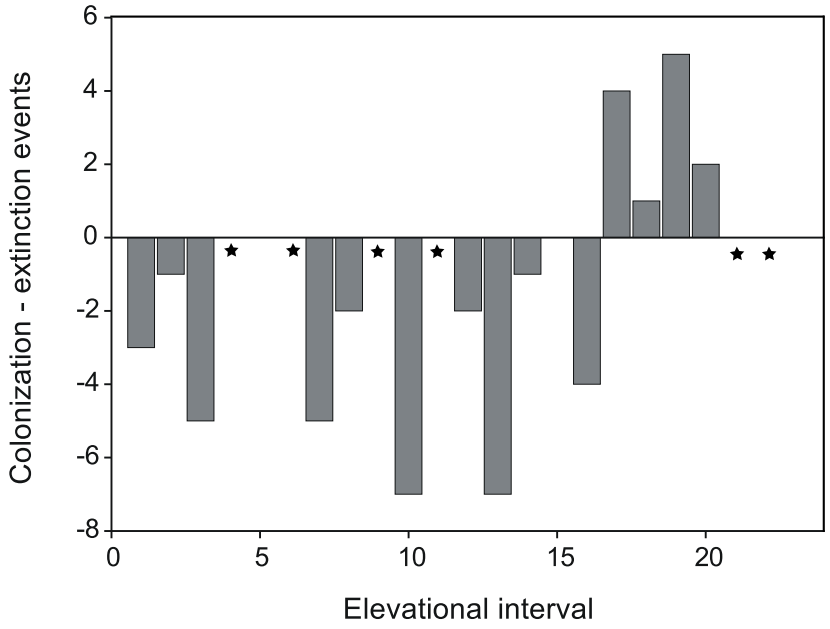

Figure 1.6. Differences between colonization and extinction events across elevational intervals. Colonizations refer to species present only in the 2007-2009 period and extinctions to species present in the 1988-1989 period but absent in the 2007-2009 period. No bars indicates equal number of colonization and extinction events, while the presence of stars represents elevational ranges for which there was no sampling sites in at least one of the two periods (see text for details).

DISCUSSION

Our comparison of historical (1988-1989) and recent (2007-2009) re-surveyed distributional data of bumblebees provides the first evidence of elevational changes in bumblebee assemblages at both species and community level. Most species shifted uphill their upper- or lower-elevation boundaries, resulting in narrower elevational ranges from one period to another. Changes in elevational ranges were, however, mainly related to increases in the lower limit of species

distribution, rather than to decreases in their upper elevational limit. Furthermore, while extinction events were especially relevant at lower and medium elevations, communities located at upper locations had a generalized positive net outcome between colonization and extinction events. In combination, all these changes over two decades in the elevational distribution patterns of bumblebee in our study region have resulted in the homogenization of species assemblages along the elevational gradient, characterised by a comparable composition between communities populating medium (900-1500 m) and upper (>1500 m) elevations.

It is increasingly suggested that tracking for climate optimum drives elevational shifts of many species in mountain areas as a consequence of upward displacements of climate conditions to which they are adapted (Pounds et al. 1999, Lenoir et al. 2008, Chen et al. 2009). Climate data from meteorological stations demonstrate that temperatures in the study region have risen by *ca.* 0.8°C in comparison with the last decade (1971–2000), whilst precipitation during this period is similar to that in recent years (IPCC 2007, Álvarez et al. 2009). Accordingly, given that bumblebees are cold-adapted species mainly related to higher latitudes and cool conditions, we suggest that increases in both average elevation and lower boundaries of species distribution might be at least partially explained by climate warming. Indeed, climate (and especially temperature) tracking has already been suggested to drive northwards range expansions of some bumblebee species such as *B. terrestris* and *B. lapidarius* in Western Europe (Macdonald 2001). However, despite some species showed elevational upwards in conjunction with raising temperatures, other species showed very little shift in their range and others even showed downward migrations as found in other studies with other animal groups (Parmesan and Yohe 2003, Hickling et al. 2006, Chen et al. 2009). This suggests that individual species' realized response to climate change might depend upon species-specific attributes that condition the capacity of different species to track rapid climate change via range shifts (Angert et al. 2011). In this line, it has been proposed that bumblebee species with a greater climatic specialization would be more susceptible to decline (Williams et al. 2007, 2009). This line of research should be a high priority in future studies as it

is fundamental to both project future distributions under various scenarios of environmental change (e.g., Stefanescu et al. 2011) and, from a conservation point of view, to identify which species are likely to exhibit range shifts.

The upward shift observed in the community composition parallels elevational and latitudinal changes at the community-level of other animal groups lagging behind climate change (Wilson et al. 2007, Devictor et al. 2012). Previous studies have found biological communities to be increasingly dominated by generalist species after changes in distribution related to climate change (Warren et al. 2001, Wilson et al. 2007, González-Megías et al. 2008). For bumblebees, differences in the tongue length influences dietary preferences, since long-tongued species tends to visit flowers with deeper corollas (Pyke 1982, Goulson and Darvill 2004, Goulson et al. 2005). Recent studies have found similar results in bumblebee assemblages in Sweden and Denmark that become increasingly dominated by short-tongued species, while long-tongued species decreased (Bommarco et al. 2011, Dupont et al. 2011). In this study, short-tongued, generalist species such as *B. terrestris*, *B. soroeensis*, *B. lucorum* and *B. lapidarius* (Goulson and Darvill 2004, Goulson et al. 2005, Goulson et al. 2008*a*) become slightly more abundant after two decades, specially at medium and higher elevations. In contrast, species that were previously common in the study region are now less widespread. Most of these species were medium and long-tongued species such as *B. sylvarum*, *B. ruderatus*, *B. ruderarius*, *B. pascuorum* and *B. hortorum* (Goulson et al. 2005, Goulson et al. 2008*a*). Furthermore, the homogenization of bumblebee communities at medium and higher elevations in the study region might therefore integrate a combination of a decline of mountain species and the increase of lowland species. Actually, typically mountain species that reach their southern limit of distribution in the Cantabrian Range such as *B. mesomelas* and *B. wurflenii* (Rasmont and Iserbyt 2010) showed the greatest retractions in their elevation range. The proximity of their range of distribution may have rendered them more sensitive to environmental change (Williams et al. 2007). This is in line with a biotic homogenization due to extinctions exceeding colonizations at lower and medium elevations and widespread species becoming more abundant at higher

elevations (McKinney and Lockwood 1999, Olden 2006). Overall, these results suggest that the composition of bumblebee communities are currently affected by climate change, but also reveal that communities would face major ongoing changes in structure and composition.

However, species' distributions are constrained by competition and other biotic interactions rather than environmental factors at their lower boundaries (Davis et al. 1998, Araújo and Luoto 2007). Thus, we must reject climate warming as the sole explanation for elevational displacements in bumblebee assemblages. One potential driver of change could be alterations in patterns of vegetation cover caused by human activities, which are more intense at lower and medium elevations all around the world (Nogués-Bravo et al. 2008). Transformation of the land cover surface –whether by intensification of human actions or land abandonment- could lead to bumblebee species carrying out elevational migrations in order to cope with changes in the spatial distribution of plant resources. In fact, changes in land-use are already demonstrated to be responsible for alterations in the geographical distribution of some bumblebee species in Western Europe (Goulson et al. 2005, Kosior et al. 2007) and North America (Grixti et al. 2009). In the Cantabrian Range, changes in land management for the last 20 years have been characterised by land abandonment and an increase of cattle numbers (García et al. 2005, Blanco-Fontao et al. 2011). However, in contrast to intensification of farming practices in Western Europe and North America (Carvell et al. 2006, Grixti et al. 2009), no significant changes in landscape structure and habitat composition are expected in our study region, thus vanishing the possibility to be one key factor driving changes in distribution patterns of bumblebees. Others likely factors suggested to govern patterns of widespread bumblebee decline such as level of pathogen infection (Cameron et al. 2011) and competition with domestic honeybees (*Apis melifera*) and other bee species (Goulson and Sparrow 2009, Thomson 2006) could be interacting with climate, leading to upward migrations in bumblebee distributions. However, further work is necessary in order to obtain data on pathogen prevalence and location of

beehives along the elevational gradient on the study area to confirm this hypothesis.

In summary, the current work demonstrates that bumblebee communities have experienced changes in their elevational distribution over the last 20 years in mountain areas of northern Spain. More specifically there has been an overall trend of range retraction in species distributions driven by extinctions at the lower elevational limit. Furthermore, these changes in the elevational distribution of species resulted in a significant homogenization of bumblebee assemblages along the elevational gradient between communities populating medium and upper elevations. We suggest that such changes (i.e., whether at species- or community level) strongly match with predictions based on the already demonstrated effects of drivers of global change on geographical displacements of bumblebee species. However, we acknowledge that further research is needed to establish the relationship between, and to discern the relative contributions of, the factors driving the alterations in bumblebee assemblages found in this study. We suggest that species biological traits such as climatic niche breadth might explain differences in bumblebee responses and should be a priority area of research. That said, it is important to note that since bumblebees are present where other insect pollinators simply cannot forage due to low temperatures, the changes in bumblebee elevational distribution outlined here could potentially affect the functioning of plant-pollinator interactions.

2 *Which species' traits determine the extent of range shifts in response to climate change?*

INTRODUCTION

Geographical and elevational species range shifts of species in relation to global warming have been widely reported (Parmesan and Yohe 2003, Hickling et al. 2006). Migration responses include a great variety of movements such as contractions and expansions of range boundaries always aimed at enabling the species to remain in suitable climatic conditions (e.g., Parmesan et al. 1999, Wilson et al. 2005, Chen et al. 2011*b*). It has been widely assumed that range shifts in species distribution are determined almost exclusively by the availability of climatically suitable habitats (Hill et al. 1999, Warren et al. 2001, Early and Sax 2011). However, the considerable variation in both the direction and the magnitude of such geographical and elevational displacements suggests that individualistic traits might interact to modulate each species' realized response (Schweiger et al. 2008, Buckley et al. 2010, Angert et al. 2011). Yet, we still lack knowledge regarding how species' traits provide a mechanistic approach for predicting species distributions under changing climatic scenarios.

Many life-history traits might limit a species' success in occupying a particular area and expanding into new ones in order to track suitable climatic conditions. For example, dispersal ability (e.g., Pöyry et al. 2009) and rate of reproduction (Angert et al. 2011) have proven to be useful determinants of the pattern of species movements. However, ecological generalism is suggested to be one of the main traits that determine response patterns in the face of climate change (Warren et al. 2001, Menéndez et al. 2007, Angert et al. 2011). Indeed, most studies have found that the relative impact of the main drivers of global change differs between specialist and generalist species (Menéndez et al. 2007, Stefanescu et al. 2011). But whilst the degree of habitat specialization and trophic specialization have been widely considered, other surrogates of ecological generalism such as climatic tolerance have been surprisingly neglected (but see Reif and Flousek 2012). This could be especially relevant for those species whose habitat suitability is mainly determined by climatic conditions, thus hampering

efforts to identify which species traits are particularly informative in predicting the effects of, and responses to, climate change on species distribution.

We investigate the role of a set of ecological and life history traits of bumblebees (*Bombus* spp.) to quantify the extent of species' range shifts over two decades in montane areas of the Cantabrian Range, NW Spain. Climate data from meteorological stations show that temperatures in the study region have risen by ca. 0.8°C between 1971-2000 (slightly less than the 0.9°C for the northern hemisphere as a whole during the same period), whilst no changes in precipitation patterns have been found (IPCC 2007). For this work we have further taken advantage of an existing dataset regarding distribution and abundance patterns of bumblebee assemblages in two episodic time periods (1988-1989 and 2007-2009) in the study region (Obeso 1992, Chapter 1). Recent findings from the comparison of bumblebee distribution patterns between both study periods show that movement responses have been species-specific, suggesting that individualistic traits might be modulating the response to the climatic warming observed in the study region (see Chapter 1). In the current work, we extend this previous study by investigating potential traits underlying such response patterns. We focused on species-specific climatic niche breadth and tongue length variation, which have been suggested to be related to bumblebee vulnerability to decline (Goulson et al. 2005, Williams et al. 2007, 2009). We also hypothesized that basic life history traits of a species may influence its response to global change (e.g., Pöyry et al. 2009), so we also considered wing morphology and species' endothermy capacity. Specifically, wing morphology has been used as a proxy for dispersal potential in insects (Simmons and Thomas 2004). We considered that these ecological traits might be useful proxies for individual species' traits such as climatic tolerance, trophic preferences and dispersal ability and in turn, for colonization and establishment success (see Angert et al. 2011 for a review).

MATERIAL AND METHODS

Study site and bumblebee data

We used historical (1988-1989) and recently (2007-2009) re-surveyed distributional data of bumblebee assemblages (Obeso 1992; see Chapter 1) in the Cantabrian Range (NW Spain). In this study, we considered a total of 82 sampling sites in 1988-1989 and 54 in 2007-2009 (Fig. 2.1). The climate of the region is Atlantic, with a mean annual temperature of 13°C and rainfall (*ca.* 1300 mm) distributed approximately evenly throughout the year. Sampling localities varied from sites located at sea level, to those at the top of mountains in the Cantabrian Range at approximately 2200 m a.s.l., thus comprising an elevational range sufficiently representative of almost all climatic conditions and suitable habitats for the regional pool of bumblebee species in our study region. In both study periods, a one-hour search was conducted at each site in a circular area of 100 m radius, during the spring and summer seasons (mid May through late August), always in maximum flower blooming. All bumblebees were identified at the species level and their abundance determined. We recorded a total of 1254 and 1692 individuals belonging to 24 and 21 bumblebee species in the first (1988-1989) and second period (2007-2009), respectively. In order to strengthen the statistical significance of our results, we restricted the analyses to those bumblebee species occurring in at least five localities during both study periods. Accordingly, twelve species satisfied the criteria for analysis (Table 2.1).

Response variables: change in local climatic space and elevational shifts between study periods

We estimated species' response to regional climate change as changes in the local climatic conditions at which a species occurred between the two study periods, as well as shifts in species upper and lower boundaries, mean elevation and

elevational extents. Local climatic space of the bumblebees was defined as the climatic conditions pertaining to their known occurrence at the sampled localities in the study region (see Kamilar and Muldoon 2010 for a similar procedure). We obtained local climate variables from the Climate Atlas of the Iberian Peninsula (Ninyerola et al. 2005). This database contains measurements of rainfall and temperature and is created by interpolating weather data from over 3000 weather stations throughout the Iberian Peninsula, at *ca.* 200 m resolution. For each species, we calculated the rate of relative change in local climatic space by comparing climatic conditions (i.e., rainfall and temperature patterns) between the two study periods. The rate of temperature change (hereafter RTC) and rate of precipitation change (RPC) was calculated as $|(1 - P_2/P_1)| \times 100$, where P_1 and P_2 are the mean value of climate variables of the sampling sites (whether mean temperature or rainfall) at which the species occurred in the first and second period, respectively (Table 2.1 and 2.2).

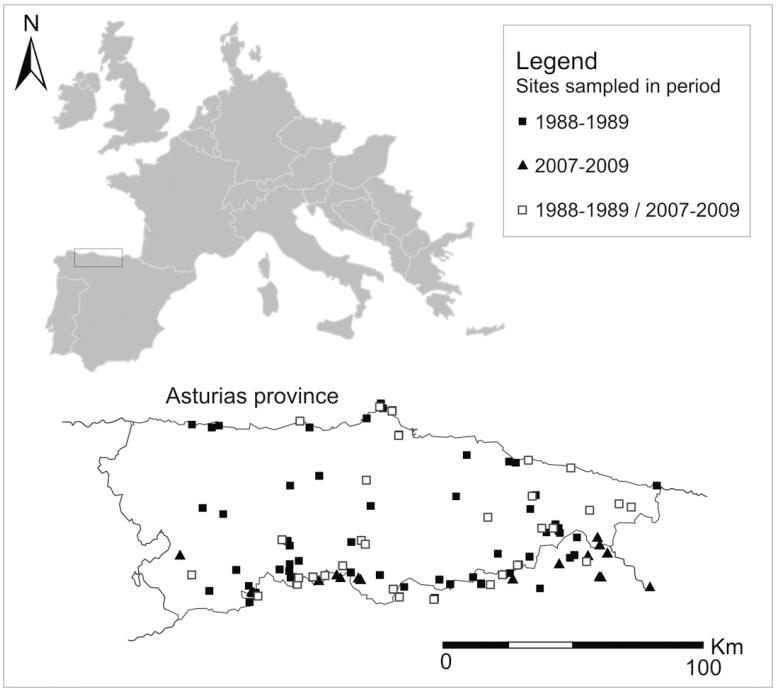

Figure 2.1. Map of the study region showing the location of the sampling localities in both 1988-1989 and 2007-2009.

Table 2.1. Mean elevation, local mean temperature (°C) and precipitation (mm^3) at which each bumblebee species occurred in 1988-1989 (P_1) and 2007-2009 (P_2) sampling periods.

Species	Mean elevation†		Local mean temperature‡		Local mean precipitation‡	
	P_1	**P_2**	**P_1**	**P_2**	**P_1**	**P_2**
B. hortorum	756.2 ± 95.2 (147, 39)	625.9 ± 161.5 (74, 17)	10.3 ± 0.04 [5.7, 14.1]	10.9 ± 0.06 [5.7, 14.0]	1058.5 ± 26.4 [771.1, 1320.0]	1069.2 ± 35.4 [834.0, 1230.1]
B. jonellus	1477.5 ± 123.3 (30, 11)	1545.4 ± 98.4 (52, 11)	7.1 ± 0.05 [8.7, 5.5]	6.8 ± 0.04 [4.9, 11.8]	997 ± 31.0 [730.2, 1346.0]	1028.2 ± 43.1 [768.0, 1346.2]
B. lapidarius	954.2 ± 172.3 (86, 16)	1485.4 ± 89.5 (154, 22)	8.9 ± 0.07 [3.7, 14.0]	6.9 ± 0.05 [3.7, 11.8]	1018.8 ± 43.3 [817.0, 1282.0]	992.4 ± 29.7 [824.0, 1230.1]
B. lucorum	1097.7 ± 110.2 (94, 30)	1470.2 ± 101.2 (222, 30)	8.9 ± 0.05 [3.7, 14.0]	7.0 ± 0.04 [3.4, 13.9]	1036.0 ± 28.0 [771.1, 1288.0]	1049.1 ± 31.1 [730.0, 1346.2]
B. mesomelas	1374.6 ± 90.0 (99, 25)	1742.2 ± 71.6 (81, 14)	7.3 ± 0.03 [0.3, 10.6]	5.6 ± 0.05 [3.7, 7.8]	981.5 ± 33.1 [797.2, 1318.0]	961.0 ± 48.2 [768.0, 1346.2]
B. muscorum	417.9 ± 78.1 (65, 31)	833.2 ± 202.2 (22, 10)	11.8 ± 0.03 [7.2,14.1]	10.1 ± 0.05 [6.5, 14.0]	1043.8 ± 31.4 [771.1, 1319.0]	1039.4 ± 47.2 [844.0, 1230.1]
B. pratorum	759.8 ± 117.7 (57, 29)	1188.8 ± 179.7 (20, 16)	10.4 ± 0.05 [0.5, 14.0]	8.3 ± 0.04 [5.4, 14.0]	1066.1 ± 30.4 [792.0, 1319.0]	1119.3 ± 37.6 [788.0, 1322.0]
B. ruderarius	1374.0 ± 113.5 (34, 14)	1511.7 ± 92.5 (104, 19)	7.5 ± 0.05 [4.9, 13.1]	6.7 ± 0.05 [3.7, 10.4]	966.7 ± 46.7 [797.2, 1288.0]	1055.2 ± 44.4 [768.0, 1334.0]
B. sichelii	1762.0 ± 89.4 (59, 12)	1620.8 ± 112.3 (131, 19)	6.4 ± 0.05 [3.6, 8.0]	6.1 ± 0.04 [3.7, 7.9]	965.0 ± 29.0 [771.1, 1288.0]	997.1 ± 55.6 [768.0, 1437.0]
B. soroeensis	1507.6 ± 90.5 (105, 19)	1613.2 ± 60.8 (368, 23)	7.8 ± 0.04 [3.7, 10.7]	6.4 ± 0.05 [3.4, 8.8]	1067.1 ± 34.4 [622.3, 1318.0]	1012.6 ± 33.2 [730.0, 1322.0]
B. terrestris	563.2 ± 115.2 (192, 36)	1059.7 ± 152.6 (278, 27)	11.1 ± 0.05 [0.3, 14.1]	9.0 ± 0.05 [3.7, 14.0]	1051.0 ± 26.7 [771.1, 1319.0]	1026.8 ± 31.7 [768.0, 1334.0]
B. wurflenii	1350.8 ± 111.4 (56, 19)	1652.4 ± 81.6 (41, 10)	8.1 ± 0.05 [0.3, 14.1]	6.2 ± 0.04 [3.7, 7.3]	986.7 ± 34.6 [771.1, 1221.0]	945.2 ± 57.1 [731.0, 1231.0]

† Between parentheses are showed the number of individuals and localities, respectively, at which species were present.
‡ Between brackets are showed maximum and minimum values.

Because the number of specimens caught at each site varied between both study periods, we used a highly conservative approach, the bootstrapping procedure, in order to increase the reliability of these measurements and to ensure that our conclusions were robust. To this end, for each species the above calculations were repeated 500 times by resampling an equal number of individuals (*ca.* 60% specimens of each individual species; see Table 1.1 in Chapter 1 and Appendix 2.1) from the 1988-1989 and 2007-2009 survey data, therefore ensuring that total sampling effort was comparable between both study periods. This procedure also allowed obtaining means (and variations) of change in RTC and RPC measures for each individual species (see Chen et al. 2011*a* for a similar procedure). All statistics we show related to RTC, RPC and elevational shifts were based on the random subsampling described above.

Table 2.2. Mean (± SE) values of the rate of temperature (RTC) and precipitation change (RPC) between 1988-1989 and 2007-2009 for each individual species. See text for details.

Species	**Rate of thermal change‡ (RTC)**	**Rate of precipitation change‡ (RPC)**
B. hortorum	5.9 ± 2.8 *	0.8 ± 2.6
B. jonellus	3.0 ± 2.2 *	1.31 ± 3.07 *
B. lapidarius	28.9 ± 2.3 ***	2.52 ± 1.5 *
B. lucorum	26.4 ± 3.4 ***	9.28 ± 2.1 ***
B. mesomelas	30.3 ± 2.8 ***	7.02 ± 3.9 *
B. muscorum	16.8 ± 2.4 **	3.69 ± 3.7 **
B. pratorum	25.3 ± 2.6 ***	2.59 ± 3.4 *
B. ruderarius	11.9 ± 2.1 *	3.81 ± 4.3 **
B. sichelii	3.7 ± 2.1	1.25 ± 0.0
B. soroeensis	1.8 ± 2.3 *	3.25 ± 2.3 **
B. terrestris	23.3 ± 2.5 ***	4.68 ± 2.1 *
B. wurflenii	30.6 ± 2.4 ***	10.30 ± 2.3 **

Significance levels are based on one-sample *t*-tests ([H_0: μ = 0 (no change)]): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Predictor variables: species' ecological traits

We used a set of species' ecological and life history traits (see Table 2.3) as explanatory variables for forecasting species-specific variation in changes in climatic space and elevational shifts between both study periods. These predictor variables included (1) climatic niche breadth, (2) endothermic capacity, (3) tongue length variation, and (4) wing morphology (wingloading and wing aspect).

In order to estimate climatic niche breadth for each species we used already available coarse-scale distribution data (i.e., continental) for west-paleartic bumblebees (Rasmont and Iserbyt 2010). Bumblebee distribution in these datasets is compiled in 50 x 50 grid cells where the presence of each individual species across West European countries is recorded. We overlayed these maps with data from the WorldClim interpolated climate surface maps (Hijmans et al. 2005) onto a GIS platform (ARCGIS 9.0, ESRI®ARCMAPTM, Redlands, California, USA). We used a 50 × 50 km spatial resolution in both distributional and climate grid-maps in order to standardize potential digitization errors. We first used principal components analysis (PCA) in order to summarize variation across climate variables between the grid cells occupied by all highly-recorded bumblebee species (i.e., all species present in at least five localities during both study periods; see above). Prior to developing the PCA, we constructed a covariation matrix among all variables and arbitrarily removed variables that displayed R^2 values greater than 0.85 in order to reduce spurious results resulting from highly correlated variables. Using this criterion, the set of variables we used were *annual mean temperature*, *annual temperature range* (maximum temperature of the warmest month minus minimum temperature of coldest month), *mean temperature of warmest quarter*, *mean temperature of coldest quarter*, *mean annual precipitation*, *mean precipitation of the wettest quarter and mean precipitation of the driest quarter* (see Table 2.4). Then, for each individual species we estimated the upper and lower limits of the climatic niche as the 2.5% and 97.5% quantiles, respectively, from the collection of eigenvectors obtained for each bumblebee species. Quantiles were used rather than absolute maximum and minimum in

order to reduce the impact of outliers which could potentially have been caused by errors in geo-referencing or digitizing (see Feeley and Silman 2010 for a similar procedure). Finally, we used the difference between the 97.5% and 2.5% quantiles to estimate climatic niche breadth for each individual bumblebee species.

We estimated species-specific endothermic capacity as temperature excess [T_{ex} = difference between thoracic temperature (T_{Th}) and ambient temperature (T_a)] of foraging bumblebees (see Verdú et al. 2007 for a similar procedure). We measured thoracic and ambient temperature of foraging bumblebees with a thermal (infrared) imaging camera (Flir i7, Flir Systems Inc., OR, USA), with an accuracy of 0.1°C. All temperatures were recorded between 09.00 and 17.00 h within 1-3 s of each bumblebee briefly landing on a flower. A total of 141 measurements of thorax temperature were obtained in the field.

We used the length of the tongue of each bumblebee species as this trait plays an important role in bumblebees, determining the range of flower types they can visit (Inouye 1978, Pyke 1982, Carvell et al. 2006). Tongue length is defined as the total length of prementum and glossa, following Prys-Jones and Corbet (1991). Wingloading was estimated as the ratio between body mass and wing area (Dudley and Ellington 1990) while wing aspect ratio (AR) was defined as $AR = 4R^2/S$, where S is the total wing area and R is the single wing length (Usherwood and Ellington 2002). The total mass of each insect was first measured to ± 0.1 mg. Both wing pairs were cut from the body and mouthparts were measured as described in Ranta et al. (1984). Tongues and wings were scanned and tongue length, wing length and wing area were measured to the nearest 0.01 mm in Image J (Schneider et al. 2012). We obtained tongue lengths and wing measurements from at least 10 workers of each species sampled across sites and elevations in years 2009 and -2010 (Table 2.3). We calculated analysis of variance (ANOVA)-based repeatabilities (R_A) of morphological measurements (tongue length: $R_A = 0.99$, $P < 0.001$, $n = 34$ repeated measures; wing length: $R_A = 0.95$, $P < 0.001$, $n = 35$ repeated measures, Nakagawa and Schielzeth 2010).

Table 2.3. Mean values of the ecological traits for each individual species. See text for details.

Species	PC1‡† (Thermal niche breadth)	PC2‡† (Rainfall niche breadth)	T_{ex} (Temperature excess)	Tongue length (mm) ¥	Wingloading (mg cm^{-2}) ¥	Wing aspect ¥
B. hortorum	6.2 [-3.5, 3.5] (1059)	5.5 [-2.7, 2.8] (1059)	---	11.73 ± 0.64 (16)	54.65 ± 1.92 (27)	12.61 ± 0.15 (27)
B. jonellus	5.4 [-3.9, 1.5] (908)	6.9 [-3.9, 3.8] (908)	9.14 ± 0.74 (14)	6.60 ± 0.28 (27)	49.97 ± 1.77 (12)	12.35 ± 0.25 (12)
B. lapidarius	6.4 [-3.0, 3.3] (976)	4.8 [-1.6, 3.2] (976)	8.58 ± 1.27 (5)	6.67 ± 0.13 (62)	53.06 ± 1.24 (26)	12.90 ± 0.15 (26)
B. lucorum	6.8 [-4.2, 2.6] (1186)	7.0 [-2.9, 4.1] (1186)	12.60 ± 0.96 (12)	6.40 ± 0.16 (46)	49.10 ± 1.51 (29)	12.34 ± 0.36 (29)
B. mesomelas	7.7 [-4.5, 3.1] (151)	3.1 [-0.6, 2.5] (151)	---	8.16 ± 0.18 (57)	51.90 ± 1.87 (18)	12.74 ± 0.14 (18)
B. muscorum	6.0 [-2.8, 3.1] (510)	4.6 [-1.3, 3.2] (510)	7.90 ± 1.55 (5)	7.66 ± 0.16 (28)	51.89 ± 1.32 (12)	12.28 ± 0.11 (12)
B. pratorum	7.7 [-4.0, 3.6] (1204)	6.9 [-2.8, 4.1] (1204)	12.43 ± 1.26 (7)	7.11 ± 0.29 (14)	48.22 ± 2.22 (10)	13.37 ± 0.29 (10)
B. ruderarius	5.7 [-2.9, 2.7] (673)	4.3 [-1.5, 2.8] (673)	9.21 ± 0.76 (13)	8.73 ± 0.12 (88)	49.23 ± 2.18 (10)	12.99 ± 0.15 (10)
B. sichelii	5.6 [-3.9, 2.0] (42)	4.5 [-2.9, 2.7] (42)	8.91 ± 0.59 (14)	5.91 ± 0.09 (82)	56.73 ± 1.77 (23)	12.90 ± 0.12 (23)
B. soroeensis	6.9 [-4.3, 2.6] (959)	6.9 [-2.6, 4.3] (959)	10.53 ± 0.31 (53)	6.96 ± 0.08 (141)	52.60 ± 1.30 (37)	12.67 ± 0.10 (37)
B. terrestris	6.5 [-2.4, 4.0] (949)	4.2 [-1.3, 2.8] (949)	---	8.52 ± 0.12 (82)	38.61 ± 0.71 (26)	12.68 ± 0.13 (26)
B. wurflenii	7.2 [-4.9, 2.3] (444)	7.5 [-2.1, 5.3] (444)	10.21 ± 0.80 (13)	7.72 ± 0.13 (72)	58.08 ± 1.56 (23)	13.01 ± 0.11 (23)

† The number of 50 × 50 km cell grids from distribution maps where each species was present (Rasmont et al. 2000) are shown in parentheses.
‡ Extreme (minimum and maximum) values are shown in brackets.
¥ The number of individuals used to estimate tongue length and wing measurements are given in parentheses.

Phylogenetic constraints

Phylogenetic constraints is an important issue when analyzing closely related species. This is because close species can occupy similar ecological niches and this in turn might cause pseudoreplication constraints due to non-independent responses between species (Losos 2008). Accordingly, we controlled for the existence of phylogenetic signals in the species' traits of West European bumblebees included in this study. To do this, we first developed a phylogenetic distance matrix including all bumblebee species using the phylogeny reported in Cameron et al. (2007). Distance matrix for each species traits were calculated from pairwise comparisons between species. Finally, by confronting both phylogenetic and each distance matrices, we were able to examine how well each species' trait was correlated with the phylogeny. A similar approach was used to test the potential phylogeny (see Kamilar and Muldoon 2010 for a similar approach).

Statistical analysis

We used simple linear or quadratic regressions to test the influence of species' traits on the relative change in both thermal (RTC) and rainfall (RPC) conditions and shifts in elevation at which different species occurred between the first and second study period (residuals were normally distributed, Kolmogorov-Smirnov tests, $P > 0.05$). We excluded endothermic capacity (temperature excess, T_{ex}) from the analysis to avoid multicollinearity; all remaining correlations among predictor variables were < 0.7 (Appendix 2.2).

The correlation between phylogenetic and distance matrices for ecological traits was performed using a Mantel's test (Package *vegan* version 1.16-32; Oksanen et al. 2011) under R software (R Development Core Team 2012). Analyses to obtain the phylogenic distance matrix were conducted using the Kimura 2-parameter model (Kimura 1980) and MEGA5 (Tamura et al. 2011). Means are shown along with one degree of standard error throughout text and tables.

RESULTS

Two eigenvectors for the set of climate variables were judged meaningful by the PCA and explained *ca.* 95% of the total variance in the set of temperature and rainfall patterns (Table 2.4). The first eigenvector (PC1) was mainly related to temperature patterns representing a positive gradient in annual mean temperature, annual temperature range, mean temperature of warmest quarter and mean temperature of coldest quarter. The second eigenvector (PC2) was mainly related to rainfall patterns, representing a positive gradient of mean annual precipitation, mean precipitation of the wettest quarter and mean precipitation of the driest quarter. We therefore used PC1 as a surrogate of thermal niche space, and PC2 of rainfall niche space at the continental scale of bumblebee distribution.

Table 2.4. Results of principal component analysis used to integrate climatic variables and estimate climate niche breadth. Main descriptors for each the two main principal components (PC1 and PC2) are highlighted in bold. Cumulative explained variation (%) is also showed.

	PC1	**PC2**
Cummulative explained variation (%)	57.1	94.4
Principal component structure		
Annual mean temp.	**0.43**	0.29
Temp. annual range	**0.48**	0.12
Mean temp. warmest quarter	**0.48**	0.12
Mean temp. coldest quarter	**0.37**	0.37
Mean annual precipitation	-0.26	**0.52**
Mean precipitation of the wettest quarter	-0.25	**0.49**
Mean precipitation of the driest quarter	-0.26	**0.47**

Mantel's test showed no significant correlation between phylogenetic and distance matrices from ecological traits considered (Mantel statistic r = - 0.001, $P > 0.05$ based on 999 permutations in all cases). This suggests the absence of any phylogenetic signal in the species' traits, thereby validating the assumption that the patterns of response we found in subsequent analysis were independent of any potential ties of kinship between bumblebee species.

RTC was positively and significantly related to thermal niche breadth (linear regression $R^2 = 0.67$; $F_{1,10} = 20.37$; $P = 0.001$; Fig. 2.2a) but no to rainfall niche breadth, tongue length, endothermic capacity and wing measures ($R^2 < 0.10$ and $P > 0.05$ in all cases). On the other hand, RPC was significantly related to rainfall niche breadth (quadratic regression $R^2 = 0.53$; $F_{2,9} = 5.10$; $P = 0.013$; Fig. 2.2b) and not to other ecological traits ($R^2 < 0.10$ and $P > 0.05$ in all cases). RTC was also negatively related to shifts in elevational range (linear regression $R^2 = 0.35$; $F_{1,10} = 5.33$; $P = 0.043$; Fig. 2.3).

We found a significant relationship between RTC and RPC (linear regression $R^2 = 0.50$; $F_{1,10} = 10.02$; $P = 0.01$) but not between thermal and rainfall niche breadth ($R^2 = 0.04$; $F_{1,10} = 0.40$; $P = 0.540$) suggesting that the species showing stronger changes in temperature patterns also showed stronger changes in precipitation patterns from one period to another, irrespective of their climatic tolerance.

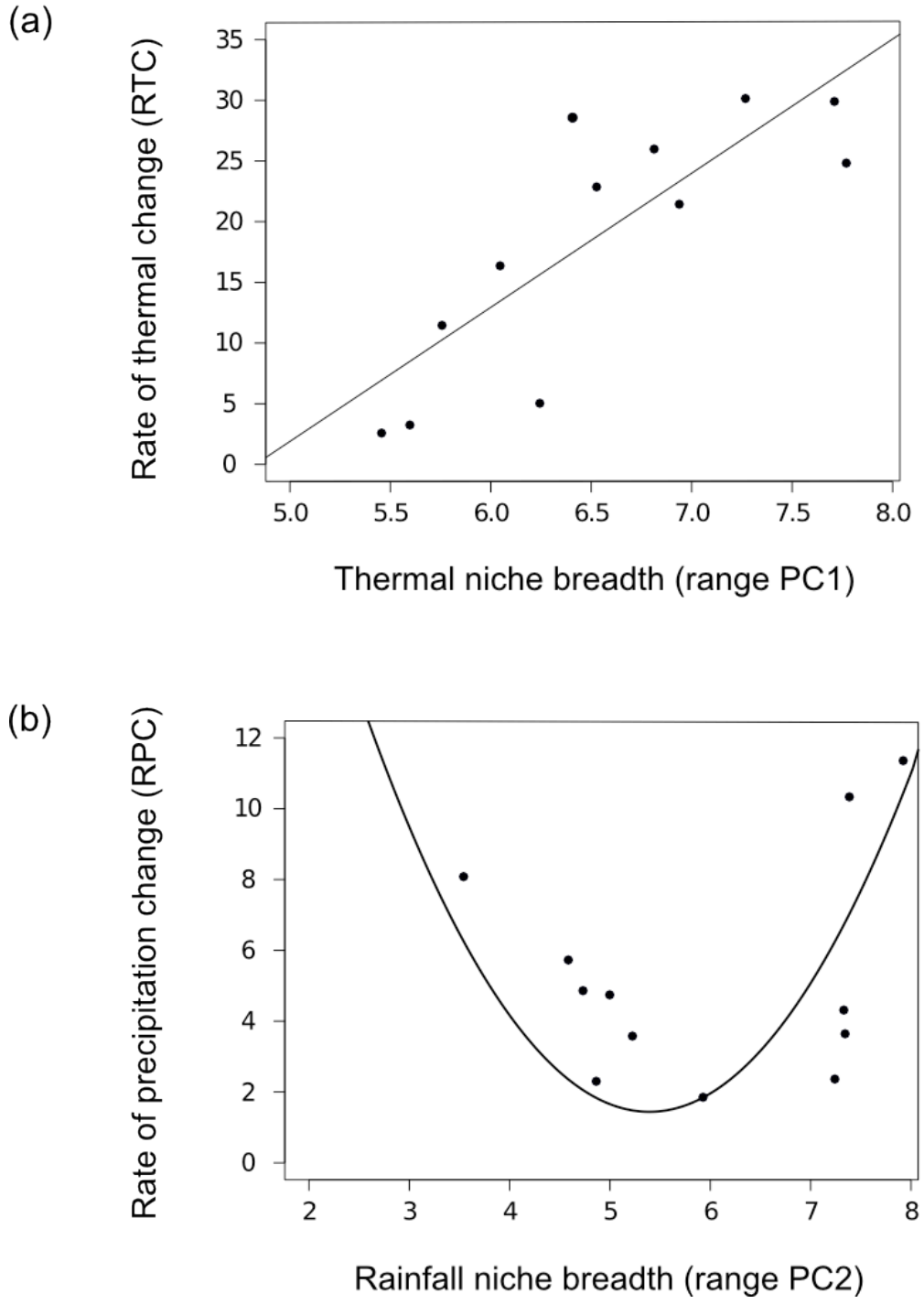

Figure 2.2. In (a) relationship between thermal niche breadth of bumblebee species and rate of temperature change (RTC). In (b), relationship between rainfall niche breadth and rate of precipitation change (RPC) (see text for details).

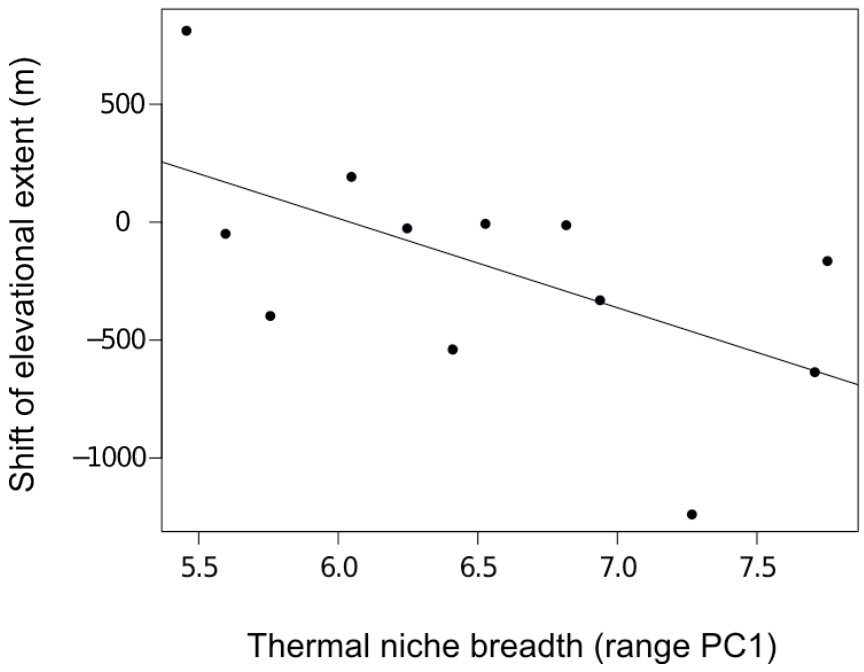

Figure 2.3. Relationship between thermal niche breadth of bumblebee species and change in elevational extent between the two study periods. See text for details.

DISCUSSION

We examined the role of a set of ecological species traits for forecasting variation in responses of bumblebee species in the face of regional climate change at the Cantabrian Range. We found that climatic niche breadth, but not other species' traits such as trophic preferences and dispersal ability, determined the rate of each individual species' realized response to climate change. Thus, bumblebee species with broad climatic tolerance showed greater changes from one period to another in the climatic conditions at which they occurred in a time span of 20-years. Accordingly, our findings suggest that climatic niche breadth might be a

particularly informative ecological trait for understanding and predicting the response of bumblebee species to climate change in mountain regions and, in turn, their distribution range shifts.

This finding is in line with previous works suggesting that climatic tolerance might be a key species trait influencing the ability of bumblebee species to cope with ongoing global change (Williams et al. 2007, 2009). The response of each individual species was, however, species-specific, since the broader the climatic niche of the individual species, the higher the degree of change in climatic conditions at which each species occurred from one period to another. It should be noted, however, that thermal niche breadth, rather than rainfall niche breadth, appeared to be much more relevant at driving the relative role of climatic niche breadth. This might be related to the narrower range of thermal conditions at which different species occur throughout their continental range, thus conferring thermal conditions a leading role in both determining a species distribution as well as modulating the rate of their response. Indeed, in line with this suggestion, the maximum rate of temperature change (RTC) was three times higher than that of precipitation change (RPC) (Table 2.2). Consistent with this, all bumblebee species (except for *B. hortorum*) tracked localities *ca.* 1.3°C cooler from one period to another, while no trends were observed for precipitation patterns in bumblebees' response (Table 2.1).

The neglect of climate-envelope models approaches is probably related to the lack of high-resolution climate data, especially at broad spatial scales. With increasingly fine-scale datasets throughout the entire range of a species distribution, such as those we used in this study, the establishment of the actual climate niche space, and in turn, climatic tolerance of each individual species, is increasingly feasible (e.g., Kamilar and Muldoon 2010). Indeed, high-resolution climate and landscape data sets coupled with more accurate information regarding temporal changes in species distribution is considered to be one of the more promising ecological tools to describe, analyze and predict species-specific range shifts in response to global environmental change (Angert et al. 2010). In this way,

our study provides a significant step forward for forecasting range shifts through the use of this mechanistic approach that is readily feasible for an increasingly number of taxa, and it is therefore an area for future research.

Tongue length and other species traits such as dispersal ability and endothermic capacity were not behind each species' realized response, suggesting the leading role of climate change on species distribution shifts against other potential environmental changes such as alterations in food-resource availability (Stefanescu et al. 2011). Indeed, this conclusion concurs with recent findings from the same study system which show that bumblebee distribution and abundance in our study region is mainly determined by climatic variables rather than by any other environmental factor such as habitat structure and composition (see Chapter 3). In any case, another explanation which cannot be discounted is the narrow range in tongue length, dispersal abilities and endotermic capacity between bumblebee species we studied. For example, although the real range in tongue length varied from 5.9 mm to 11.7 mm, the 75th percentile included species with tongue lengths between 5.9 and 8.4 mm, suggesting a range not wide enough to quantify the influence of species' trophic niche breadth on range shifts. Indeed, when we reanalysed our data reducing the range of climatic niche breadth and classifying species into two levels: generalist vs. specialist, the effect of climatic niche breadth on the rate of species' response become non significant (data not shown). Accordingly, the use of continuous measures in describing species traits, rather than integrating species into discrete groups might be a more valuable way to describe species traits and should, we feel, be considered for future research. Otherwise, establishing a deterministic relationship between life history traits and the extent of species response to climate change (or any other environmental change) will remain elusive.

Conclusions

Our findings suggest that climatic niche breadth strongly determined the responses of bumblebee species in the face of climate warming registered in our study region. We emphasize, however, that this conclusion might be particularly generalizable to those species whose habitat suitability is strongly determined by climatic conditions, as it is the case of bumblebee species. This is because, at least in our study system, the relative role and suitability of other species traits, might be overridden by the strong effect of climate in species distribution and abundance. Indeed, in other study systems, trophic preferences has successfully been proven to influence a species' response in habitats undergoing climate change (e.g., Stefanescu et al. 2011). We would therefore suggest that detailed information on a species' biology and natural history is crucial for determining the fundamental traits underlying the species' responses to climate warming and any other global environmental change. Furthermore, given that the relative role of any species trait is species-specific, potential idiosyncrasy in each individual species response (even when considering closely related species) should therefore also be considered for forecasting variations in the response of different species to climate change. Taken together these theoretical guidelines may help to understand which species' traits provide more informative and useful insights for predicting variations across each individual species' realized response to climate change.

3

Determining habitat suitability of mountain bumblebees: a baseline approach for testing the impact of climate change on species presence and distribution

INTRODUCTION

Accurate information regarding species' habitat suitability is widely recognized as fundamental both to establish potential geographic ranges of species and to project future distributions under various scenarios of environmental change (Thomas et al. 2004, Soberón 2007, Stefanescu et al. 2007, Wiens et al. 2009). Determining habitat suitability also has incentives for biodiversity conservation with potential benefit to conservation biologists and natural resource managers, as it provides key guidelines to prioritize where and how we should act to efficiently sustain favourable environmental conditions for species persistence (Hannah et al. 2002). Climate-envelope approaches (i.e., those integrating both species distribution and climate data) have been considered critical both to determine species' geographic ranges and to predict where a species could be found in the face of global climate change (e.g., Hijmans and Graham 2006, Araújo and Luoto 2007, Loarie et al. 2008). However, other interacting factors such as habitat and food-resource availability could determine the species distribution and restrict it from occupying particular areas. This might be especially true in mountain regions where topography strongly modifies macroclimatic patterns at small spatial extents (Trivedi et al. 2008, Gutiérrez-Illán et al. 2010). Yet, we still lack detailed knowledge on how environmental factors in mountain ecosystems interact to determine the distribution and abundance of species and how we could use these data as an ecological framework for testing the impact of climate change.

It is increasingly suggested that mountain systems worldwide are especially prone to experience the impact of changing climatic conditions (e.g., Nogués-Bravo et al. 2007, Stefanescu et al. 2011) and hence, they should be a high priority in ecological and biogeographical studies in order to conserve mountain biodiversity. Climate warming is increasingly suggested as an important environmental driver behind elevational range shifts in both plant (e.g., Lenoir et al. 2008, Jump et al. 2011) and animal taxa (Konvicka et al. 2003, Chen et al. 2009) in mountain systems. Nevertheless, studies examining elevational shifts of species in

conjunction with changing climatic conditions have consistently found that movement responses are strongly variable between species (La Sorte and Thompson 2007, Lenoir et al. 2010, Crimmins et al. 2011), while others have even found poor fits of climatic models on species distribution (e.g., Trivedi et al. 2008). Such findings suggest that environmental factors other than climate might interact to modulate each individual species' realized response to climate change (Buckley et al. 2010, Angert et al. 2011). In highly mobile species (i.e., flying insects) inhabiting mountain areas, we can expect that small changes in climate at local scales could cascade into strong modifications of species distribution even when such changes are produced at short time-spans (e.g., Wilson et al. 2007, Gutiérrez-Illán et al. 2010). Accordingly, disentangling the set of environmental factors that determine habitat suitability (while considering the potential individual species' response) is key for understanding and predicting species' distribution patterns as well as for evaluating their response to climate change (Hampe 2004, Araújo and Luoto 2007, Luoto et al. 2007, Gutierrez-Illán et al. 2010).

Here, we investigated the role of both topoclimate and land-cover variables on bumblebee species distribution and abundance in the Cantabrian Range, NW Spain. Bumblebees are social bees of the genus *Bombus* that exist primarily in temperate, alpine and artic zones throughout the Northen Hemisphere but become scarce in southern climates (Goulson 2010). The Cantabrian Range is a highly diverse ecotonal zone between the Eurosiberian and the Mediterranean regions in Europe, which harbours a total of 24 bumblebee species from boreal, temperate and mediterranean biogeographical regions (Obeso 1992). Climate data from meteorological stations shows that this region is subject to climate warming (regional temperature has risen by *ca.* 0.8°C since 1970 to 2001) whilst no changes in precipitation have been found (IPCC 2007). This temperature increase has resulted in an upward shift of isotherms of *ca.* 160 m based on the regional altitudinal temperature lapse rate of -0.5°C 100 m^{-1} (Mateo 1970). Recent findings showed that, despite most bumblebee species have performed uphill shifts in conjunction to that of regional climate warming over the last two decades, the direction and magnitude in the observed elevational range shifts were species-

specific (see Chapter 1). This suggests that climate, but also other life history traits such as habitat preferences, might determine the spatial patterns of species distributions in our study region, and in turn, species vulnerability to changing climatic conditions. To investigate this prediction, we explicitly evaluate the role of climate at explaining the bumblebee species distribution (i.e., presence and absence) and abundance as well as other factors that contribute to habitat suitability (topographic and habitat-cover variables). These factors (when adequate) were analyzed at increasingly nested neighbourhood distances (100 - 3000 m) as different bumblebee species may respond at different spatial scales (e.g., Steffan-Dewenter et al. 2001, 2002). We also used an additional historical dataset from 1988-1989 (Obeso 1992) both to calibrate our current distribution and abundance models and to test predictions on the relative change that has occurred over the last two decades. Specifically, we addressed whether current estimated distribution and abundance were congruent with historical bumblebee distribution patterns (thus validating model's consistency) and evaluated the role of climate and landscape context at explaining potential changes in species distribution and abundance between both study periods.

MATERIAL AND METHODS

Study site and bumblebee data collection

This study took place between 2007 and 2009 at the Cantabrian Range, NW Spain (42.8–43.5° N and 4.5–7.1° W; Fig. 3.1a). The climate of the region is Atlantic. Annual average temperature is *ca*. 13°C and total precipitation is 1250 mm. The current regional landscape might be considered as highly variegated (*sensu* McIntyre and Hobbs 1999), containing remnant forest fragments standing out from a non-forest matrix mainly composed of pastures, heathlands in abandoned meadows and scattered small villages (García et al. 2005). A total of 55 localities, mostly in the provinces of Asturias and León, were located along almost the entire altitudinal gradient occurring in the study region (0 - 2200 m a.s.l.). The spatial

framework we used therefore included almost all habitat types and configurations potentially present across the elevational gradient of species distribution, acting as a good proxy of the current landscape throughout the Cantabrian Range (García et al. 2005). The regional landscape ranged from conifer plantations [mainly afforestations of *Pinus* spp. (Pinaceae) and *Eucaliptus* spp. (Myrtaceae)] and mixed deciduous forests [including secondary-growth forests with species such as *Ilex aquifolium* (Aquifoliaceae), *Sorbus aucuparia* (Rosaceae) and *Crataegus monogyna* (Rosaceae)] in mid- and low-lands to shrubs and subalpine meadows [mainly *Fagus sylvatica* (Fagaceae), *Quercus* spp. (Fagaceae) and *Betula alba* (Betulaceae)] at upper elevations.

At each locality, we conducted standardized time-constrained transects throughout a circular area of 100 m radius. Each locality was visited once and sampling was continued until 30 min was spent at that site by two collectors (or 1 h by one collector). Foraging individuals were caught with a hand net and when their identification was not possible in the field, they were collected for identification in the laboratory. The identification of each bumblebee species and their caste (whether queen, worker or male) was always carried out by the same person (E.F.P). Abundance was estimated as the sum of all counts for each species at each sampling site. We sampled each locality throughout the spring and summer seasons (mid May to late August) of 2007-2009. Surveys were carried out during maximum flower blooming to avoid differences in the composition of the bumblebee assemblage between localities due to variations in food-resource availability. Samplings were always conducted in warm, dry conditions and were suspended on rainy days when bumblebees were inactive. In order to ensure that analyses include species whose distributions were representatively sampled, species such as *B. ruderatus*, *B. hypnorum*, *B. subterraneus*, *B. monticola*, *B. pascuorum*, *B. inexpectatus*, *B. mucidus* and *B. sylvarum*, which comprised together less than 5% of the total and were all caught in less than 10 localities (see Table 3.1), were excluded for subsequent analyses. Accordingly, 13 out of 21 bumblebee species actually satisfied the former criteria for analysis (Table 3.2 and 3.3).

Table 3.1. List of bumblebee (*Bombus* spp.) species caught in 55 sampling sites at the Cantabrian Range between 2007 and 2009.

	NoI		NoL	
	Historical	Current	Historical	Current
Species				
B. hortorum	147	61	39	12
B. humilis	11	85	6	14
B. hypnorum §	6	1	3	1
B. inexpectatus §	4	6	3	1
B. jonellus	30	21	9	11
B. lapidarius	86	138	9	21
B. lucorum	94	162	30	25
B. mesomelas	99	63	25	13
B. monticola §	11	15	4	6
B. mucidus §	3	21	3	6
B. muscorum	65	22	31	10
B. pascuorum §	116	21	35	7
B. pratorum	57	16	29	11
B. ruderarius	34	103	14	19
B. ruderatus §	17	3	7	3
B. sichelii	59	98	12	17
B. soroeensis	105	314	19	23
B. subterraneus §	5	3	2	2
B. sylvarum	46	8	19	6
B. terrestris	192	69	36	19
B. wurflenii	56	41	19	10

§ Species which were excluded due to the low number of caught individuals and localities where they were present (see main text for details).
NoI = number of individuals of each species caught between 1988-1989 (historical) and 2007-2009 (current) sampling periods.
NoL = number of localities at which individual species was present in the same two sampling periods.

Predictor variables

The distribution and abundance of bumblebee species were examined in relation to a set of independent environmental variables as a proxy of species-based habitat suitability (e.g., Steffan-Dewenter et al. 2001, 2002, Krauss et al. 2003, Gutierrez-Illán et al. 2010). We arbitrarily integrated slope, temperature and rainfall patterns as topoclimatic variables, and habitat configuration and composition as land-cover variables. The effects of some variables were also analyzed at increasing neighbourhood distances. Using a geographic information

system (GIS), we established non-overlapping circular areas at increasingly wider scales from the centre of each point where we carried out the sampling of bumblebees within each locality (see *Bumblebee data collection* above). This approach has been successfully used in previous studies to measure the diversity and abundance of species associated with landscape context at multiple spatial scales, especially insect species (e.g., Steffan-Dewenter et al. 2001, 2002). A total of seven radii of 100, 250, 500, 750, 1500, 2000 and 3000 m were established, which represented a nested set of spatial scales (Fig. 3.1b). These scales were chosen because they are similar to known flight and foraging distances of most bumblebee species, some of them occurring within the regional pool of species such as *B. terrestris*, *B. ruderatus* and *B. hortorum* (Hagen et al. 2011).

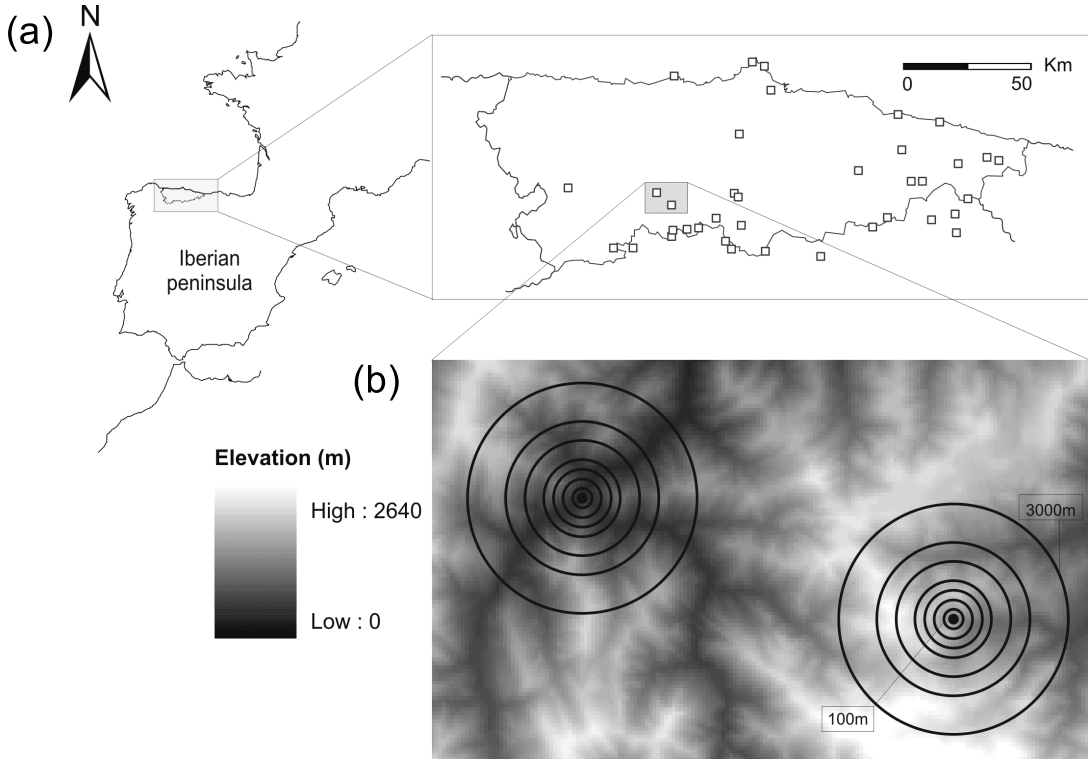

Figure 3.1. In (a), map of the study region showing the location of sampled sites for bumblebee surveys between 2007 and 2009 in the Cantabrian Range. In (b), is showed a detailed view of two sampled sites with circular buffers of 100, 250, 500, 750, 1500, 2000 and 3000 m from the centre of sampling areas (see text for details). Shaded areas indicate different altitudes extracted from a Digital Elevation Model (DEM) derived from a geographic information system (GIS) of the study region.

Topoclimatic variables

This group of variables induced temperature (i.e., linear and quadratic fit), precipitation and mean slope at 100 m and 3000 m spatial scale. Initially, we also included elevation as predictor variable, although it was finally excluded from our analysis because of the high degree of correlation with temperature ($|r| = 0.99$; Appendix 3.1). Climatic variables (i.e., linear and quadratic temperature and rainfall) of each sampling locality were extracted from the Climate Atlas of the Iberian Peninsula (Ninyerola et al. 2005; http://opengis.uab.es/wms/iberia/). This database contains various measures of rainfall and temperature patterns and is created by interpolating weather data from over 3000 weather stations throughout the Iberian Peninsula at *ca*. 200 m resolution. We focused on temperature and rainfall given that these variables play a key role in the species distribution in mountain systems where there are strong thermal and humidity gradients with elevation (e.g., Gutierrez-Illán et al. 2010, Stefanescu et al. 2011). Mean slope at 100 and 3000 m circular areas from sampling sites were extracted by overlapping into a GIS both the seven nested spatial scales and a Digital Elevation Model (DEM) from the study site (Fig. 3.1b). Mean slope at 100 and 3000 m (i.e., the lower and upper spatial scales) were used as they might act as a good proxy of the roughness of each locality where mean elevation is likely to be scale-dependent (see Fig. 3.1b). Accordingly, they could have important effects on the distribution and abundance of bumblebee species in mountain ecosystems because of their role in determining the degree of site isolation.

Land-cover variables

Land-cover variables included the percentage of all habitat types that appeared at each of the seven nested spatial scales located at sampling sites (see Fig. 3.1b), thus determining their composition and vegetation structure. We used the data from the second (1997-2007) Spanish National Forest Inventory (SNFI), which includes an accurate typological classification of vegetation structures of the study region

(MMA-DGB 2005). Because of the high variety of cover types potentially occurring in the SNFI (for example, there are more than 20 forest structural types based on species composition; MMA-DGB 2005), we simplified and integrated land-cover types into broader groups following the recommendation of Steffan-Dewenter et al. (2002). We initially defined six groups of land-cover variables describing the habitat types (i.e., grassland, forest, shrublands, rocks) as well as other potential indicators of landscape configuration (i.e., mean habitat size and habitat isolation; Krauss et al. 2003). However, these parameters were highly correlated with other landscape variables (i.e., we discarded land-cover variables when they achieve pair-wise correlations $|r| > 0.70$; Appendix 3.1) or were present on fewer than 10% of each of the seven nested spatial scales (data not shown). Hence, they were excluded from our analysis and the final list of variables list included the percentage of grasslands (coded as *grassland*), forest cover (*forest*), shrublands (*shrubland*) and the Shannon-Wiener diversity index of land-covers (*H*).

For each landscape area we thus determined the percentage of *grassland* that included natural, seminatural, intensive and extensive pasture lands, all of which were expected to represent a land-cover type especially suitable for bumblebee species (see Krauss et al. 2003 for a similar approach in butterfly species). The percentage of *forest*, which integrated coniferous stands, deciduous forest and secondary-growth forest species because of they potentially influence landscape connectivity for bumblebees (Kreyer et al. 2004). *Shrubland* included all shrub species in the study region such as *Genista* spp., Calluna *vulgaris* and *Erica* spp. and provided (together with grasslands), one of the most suitable floral-resources for bumblebees in our study region (see Chapter 4). Following Thies and Tscharntke (1999) and Steffan-Dewenter et al. (2002) we also calculated *H* using the Shannon-Wiener index based on the above-mentioned three land-cover types (i.e., grassland, forest, shrubland; Krebs 1989). We decided to include this later landscape metric because of its demonstrated effect on species composition on heterogeneous landscapes , specially insects (e.g., Steffan-Dewenter et al. 2002).

Model calibration

We used Generalized Linear Models (GLM) for the presence and absence (1/0 data) and abundance (count data) of bumblebee species. GLMs fitted to dependent variables were based on either binomial distributions and logit link functions (presence data) and log-link functions (abundance data), using the *glm* and *pscl* libraries, respectively, in the R software (R Development Core Team, 2012). For each species, we generated three families of distribution and abundance models. "Topoclimatic models", which only included topoclimatic variables (i.e., linear and quadratic temperature, rainfall and mean slope at 100 m and 3000 m neighbourhood distances); "Land-cover models" which included context dependent land-cover variables (i.e., grassland, forest, shrubland and the Shannon-Wiener diversity index *H*); and "Combined models" which included both sets of environmental variables (i.e., topoclimatic and land-cover variables). In order to get comparable estimates between variables, we standardized all variables. Unless otherwise indicated, average values are reported as mean ± standard error (±1 SE) throughout the text. Following the above-described multi-scale approach (see *Predictor variables*), we checked which spatial scale better explained the distribution and abundance of bumblebee species. We thus fitted land-cover variables to univariate models of the presence and abundance of bumblebee, repeating this procedure for each land-cover variable (n = 4) and spatial scale (n = 7). This resulted into 28 univariate models for each bumblebee species and dependent variable (i.e., bumblebee presence and abundance). For each land-cover variable and bumblebee species, we thus selected the spatial scale better fitting the presence and abundance based on AUC (for presence-absence data) and Spearman (r_s) correlation (abundance) scores (see below), and we incorporated them into models (see Appendix 3.2 and 3.3).

Starting from full models that included both topoclimatic and land-cover variables (i.e., combined models), we tested all possible variable subsets and selected the "best" model among all competing models based on the Akaike's information criterion (AIC, hereafter) score. For the best model, we also checked

the spatial autocorrelation of the residuals of the GLMs by means of Moran's I tests with the *spdep* library (Bivand et al. 2012, R Development Core Team 2012) in order to test the presence of spatial aggregation between pairs of locations and spatial scales.

Model evaluation

In order to assess the predictive power of bumblebee distribution models, we calculated the receiver operation characteristic (ROC) curve and the area under the ROC curve (AUC) with the *PresenceAbsence* library (R Development Core Team 2012). The AUC provides a metric of the model's predictive power, ranging from 0.5 (null predictive power) to 1.0 (perfect prediction). For abundance data, we obtained Spearman-rank correlation coefficients (r_s) between fitted and observed values with the *Hmisc* library (R Development Core Team, 2012). We calculated AUC and r_s coefficients for the topoclimatic, land-cover and combined models.

Furthermore, all models were evaluated using *k* -fold cross-validation, a re-sampling approach to assess the robustness of measures (Hastie et al. 2009). We thus divided each species dataset into *k* independent elements, and for each of them we used *k*-1 elements to estimate the model and the *k*th element to validate it (Hastie et al. 2009). For this purpose, we randomly re-sampled 67% of the original data, calculated the parameter estimates on the best model, and used them to obtain predicted values for the remaining 33% dataset. For each cross-validated model, we finally calculated the average and standard error of AUC or r_s coefficient based on 50 realizations of the above-described cross-validation procedure.

Comparing current and past trends

Models were validated with historical (1988-1989) distributional data already available for bumblebees in our study region (Obeso 1992). In order to increase

model validity and discriminatory power, we restricted our comparison to localities sampled in 1988-1989 and 2007-2009 study periods ($n_{localities}$ = 23) and excluded those species occurring in less than ten localities in both time periods ($n_{species}$ = 10, Table 3.1). For each bumblebee species, the final objective of validations was to assess if observed and estimated measures of distribution and abundance during 2007-2009 were also congruent for the 1988-1989 period, but contingent to the environmental context sampled during the time span. For such purpose, we established a topoclimatic and land-cover scenario for the 1988-1989 study period (see below).

Climatic data from meteorological stations showed that temperatures in the region have risen 0.8°C between 1971-2000, whilst no changes in precipitation patterns have been found (IPCC 2007). On the other hand, the comparison between the first (1986-1996) and second (1997-2007) Spanish National Forest Inventory showed no significant changes in vegetation structure at least below the limits of the upper spatial scale (3000 m) located at each locality under study (author's unpublished data). Therefore, although we acknowledge that other landscape changes have probably occurred at local scales, we suggest that climate warming was the main difference in the environmental features between the first and second sampling periods. In order to validate our models, we developed datasets with the presence and abundance of each bumblebees species for 1988-1989 period, that comprised a decrease of 0.8°C (i.e., relative to 2007-2009 values for each locality), but contingent to the locality-dependent land-cover features of 2007-2009.

We were interested to compare model predictions between current (2007-2009) and historical (1988-1989) datasets (see above) and we thus test the model performance of both datasets predicting the presence/absence and abundance of bumblebee species. We proceed calculating the model estimates of both topoclimatic and land-cover variables (i.e., the two family of models separately, see *Model calibration*) with a given dataset (i.e., current or historical) and calculated its predictive power (i.e., AUC or r_s; see *Model evaluation*) based on the left-out dataset not used to calculate the previous model predictions (i.e., historical or

current, respectively). Confident limits were estimated using bootstrapping, generating separate models from random subsets of data. We randomly resampled a subset of the original data (i.e., both current or historical datasets) to obtain the 67% of the number of individuals surveyed per locality. For instance, to generate a resampled subset for the current presence/absence and abundance of *B. hortorum*, we randomly selected 37 out of 56 and 59 out of 89 individuals (i.e., 67% of original caught individuals; Table 3.1) from the current and historical datasets, respectively. Resampled individuals included the environmental variables associated to its sampled locality. After 50 realizations, we calculated the mean and standard error of the AUC and r_s and used a *t*-test to check differences in the topoclimatic and land-cover variables and its predictive power between current and historical datasets.

RESULTS

No spatial autocorrelation were found in the residuals of both bumblebee species' presence and abundance (see Appendix 3.4). Overall, the ranking of variables selected in combined models was consistent across species, with temperature (both linear and quadratic terms) as the most frequent variable included in both presence and abundance models. Other topoclimatic variables, such as rainfall and mean slope at 100 and 3000 m, had low explanatory power for predicting bumblebee presence or abundance, although this occurred especially for presence models (Table 3.2). The same was true for land-cover variables, which were more frequently selected by the "best" models of bumblebee abundance (Table 3.2 and Table 3.3).

For combined presence models, AUC values ranged from 0.70 to 0.93 (total mean 0.81 ±0.02), indicating good model performance that was corroborated by cross-validation tests (Table 3.2). Combined models had higher AUC values in all species than presence models based on topoclimatic and land-cover variables in isolation. When we compared the overall between-species performance, combined

models were significantly related to topoclimatic models (correlation between AUC values of all species r_s = 0.70, P < 0.01, n = 13) but not to land-cover models (AUC = 0.41, P > 0.05, n = 13), suggesting the leading role of topoclimatic variables within combined models. However, although presence models based on combined variables had higher model performance, both topoclimatic and land-cover models consistently gave good model performance in isolation (total mean 0.71 ± 0.03 and 0.65 ± 0.02, respectively; Table 3.2, Fig. 3.2a).

For combined abundance models, r_s ranged from -0.01 to 0.93 [total mean 0.45 ± 0.11]. Eight abundance models had r_s scores above 0.25 indicating good model performance. Overall, combined models had r_s values higher than topoclimatic or land-cover models. As occurred with overall between-species performance for presence data, combined and topoclimatic models were more accurated (r_s = 0.74, P < 0.01, n = 13) than land-cover models (r_s = 0.18, P > 0.05, n = 13). In any case, some topoclimatic and land-cover models fitted better than combined models (Table 3.3; Fig. 3.2b).

Comparing presence and abundance performance between species, there was no relationship among combined, topoclimatic and land-cover models (i.e., correlation between AUC and r_s values; r_s < 0.01, P > 0.05, n = 13), suggesting that relative model predictability was inconsistent between the two data types.

Table 3.2. Global results of Generalized Linear Models predicting the presence of bumblebees on the basis of topoclimatic (linear and quadratic temperature, precipitation, mean slope at 100 m and 3000 m buffer areas) and land-cover (forest, shrubland, grassland and the Shannon Diversity Index of Land-cover) features. We showed results corresponding to the best model (i.e., with the lowest corrected AIC), selected from combinations of variables presented in the table's heading line, and at the fitted nested spatial scale that maximised model predictability (see Appendix 3.1). Values shown include parameter estimates for the different variables, information-criterion scores (corrected AIC) of fitted models, AUC scores evaluating the predictability of the fitted model for combined (AUC), topoclimatic (AUCTop) and land-cover (AUCHab) variables. We also included random cross-validations with an independent subset of the original data (AUCCom-CV; only for combined variables; see *Model evaluation*).

	Parameter estimates											**Predictability**			**Random cross-validation**
		Topoclimatic variables					**Land-cover variables**								
Species	**Intercept**	**Temp**	**Temp²**	**Rainfall**	**Slope 100**	**Slope 3000**	**Forest**	**Shrub-land**	**Grass-land**	***H***	**AICc**	**AUC**	**AUCTop**	**AUCHab**	**AUCk**
B. hortorum	-1.36***	1.17***	-	-	-	-	-	-	0.56	-	44.6	0.78 ± 0.08	0.75 ± 0.08	0.61 ± 0.08	0.75 ± 0.11
B. humilis	-3.32***	-	1.49**	-1.42*	-1.71*	-	1.54***	-	-	1.06**	38.7	0.93 ± 0.04	0.80 ± 0.08	0.85 ± 0.05	0.84 ± 0.10
B. jonellus	-0.54	-	-1.19	-	-	-0.84*	-	-	-	-	42.9	0.76 ± 0.08	0.76 ± 0.08	0.50 ± 0.05	0.77 ± 0.13
B. lapidarius	-1.29**	-2.65***	-	-	-	-	2.44***	-	1.53**	-	42.6	0.89 ± 0.08	0.64 ± 0.09	0.64 ± 0.08	0.86 ± 0.11
B. lucorum	0.71*	-	-	-	-	-	-0.60*	-	-	0.78	53.3	0.70 ± 0.09	0.50 ± 0.08	0.65 ± 0.09	0.69 ± 0.11
B. mesomelas	-1.95**	-2.52**	-	-	-	-	-	-	1.50*	-1.93	35.1	0.91 ± 0.05	0.84 ± 0.06	0.70 ± 0.08	0.88 ± 0.09
B. muscorum	-1.42***	0.88**	-	-	-	-	-	-	-	-	41.5	0.77 ± 0.08	0.77 ± 0.07	0.50 ± 0.05	0.76 ± 0.11
B. pratorum	-2.17	-	-0.71	-	-	-	2.64	2.50	-	-	41.8	0.82 ± 0.07	0.71 ± 0.08	0.80 ± 0.07	0.77 ± 0.11
B. ruderarius	0.81*	-	-0.92*	-	-	0.68	-0.69*	-	-	-	53.3	0.75 ± 0.08	0.67 ± 0.08	0.65 ± 0.08	0.71 ± 0.09
B. sichelii	0.05	-2.24*	-2.51*	-	-	-	-	-	2.38	-	42.2	0.83 ± 0.06	0.75 ± 0.07	0.59 ± 0.09	0.80 ± 0.11
B. soroensis	2.25***	-3.94*	-5.61**	-	1.24*	-	-	-0.95	-	-	38.4	0.90 ± 0.06	0.88 ± 0.06	0.58 ± 0.09	0.80 ± 0.13
B. terrestris	-0.24	-	-	-	-	-	-	-	0.85**	-0.62	53.8	0.71 ± 0.08	0.50 ± 0.06	0.65 ± 0.06	0.72 ± 0.12
B. wurflenii	-0.25	-	-1.57*	-1.04**	-	-	-1.66**	-	-	-	38.0	0.88 ± 0.05	0.75 ± 0.09	0.77 ± 0.08	0.82 ± 0.12

H = Shannon-Wiener index of land-cover types. Significance levels: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3.3. Summarized results of Generalized Linear Models predicting the abundance of bumblebees on the basis of topoclimatic (temperature, quadratic temperature, precipitation, mean slope at 100 m and 3000 m buffer areas) and land-cover (forest, shrubland, grassland and the Shannon Diversity Index of land-cover) features. Spearman rank scores (r_s) evaluated the predictability of the fitted model for combined (*r*Com) topoclimatic (*r*Top) and land-cover (*r*Hab) variables. For further conventions, see Table 3.1.

	Parameter estimates											**Predictability**			**Random cross-validation**
		Topoclimatic variables					**Land-cover variables**								
Species	**Intercept**	**Temp**	**Temp²**	**Rainfall**	**Slope 100**	**Slope 3000**	**Forest**	**Shrub-land**	**Grass-land**	***H***	**AICc**	r_s	***r*Top**	***r*Hab**	***r*k**
B. hortorum	0.77***	2.49***	-1.15	-	-	-0.76	-	-	0.33*	-0.42***	95.4	0.20 ± 0.15	0.38 ± 0.13	0.29 ± 0.14	0.18 ± 0.10
B. humilis	-1.83***	0.60**	0.84***	-0.93***	-0.99***	-	-	-	-0.44*	-0.49***	89.9	0.98 ± 0.00	0.92 ± 0.02	0.23 ± 0.15	0.84 ± 0.06
B. jonellus	0.67	-	-2.51**	-1.05***	-	-	-	0.95	-	-	64.9	0.27 ± 0.14	0.52 ± 0.11	-0.07 ± 0.10	0.24 ± 0.10
B. lapidarius	3.45	0.44	-1.37***	-2.15***	-	-	-1.32***	-0.77**	1.36***	-0.47	174.2	-0.03 ± 0.15	0.38 ± 0.13	0.24 ± 0.15	-0.03 ± 0.08
B. lucorum	1.06***	-0.48*	-	0.24***	-0.18	-	-	0.43***	0.56***	-	208.5	0.82 ± 0.05	0.30 ± 0.14	0.80 ± 0.06	0.32 ± 0.10
B. mesomelas	-1.07**	-2.52***	-	-	-	0.43***	-	0.23	-	-	91.6	0.80 ± 0.06	0.86 ± 0.04	0.05 ± 0.15	0.67 ± 0.07
B. muscorum	1.26**	4.30***	-2.46***	-0.54*	-	-	-1.46*	-	-	-	67.8	0.14 ± 0.15	0.30 ± 0.14	0.05 ± 0.15	0.20 ± 0.08
B. pratorum	-2.20*	-	-0.66	-	-	-	2.26*	1.99	-	-	61.3	0.48 ± 0.12	0.33 ± 0.14	0.45 ± 0.12	0.39 ± 0.07
B. ruderarius	2.59***	0.90	-1.85***	-1.19***	-	0.39**	-1.54***	-	-0.63***	-0.22*	143.8	0.76 ± 0.07	0.44 ± 0.12	0.29 ± 0.14	0.03 ± 0.09
B. sichelii	-0.13	-1.38	-	-	-0.80	1.07**	-2.12***	0.38	-	1.14	128.7	-0.08 ± 0.15	-0.01 ± 0.15	0.45 ± 0.12	-0.03 ± 0.06
B. soroensis	2.66***	2.70***	2.13	-	-0.56	-	-0.42	-0.45***	-0.34***	0.66***	218.9	-0.09 ± 0.15	-0.12 ± 0.15	0.26 ± 0.14	0.16 ± 0.11
B. terrestris	0.37	-	0.32***	-0.35***	-	-	-0.24	-	-	-0.71***	139.4	0.68 ± 0.09	0.46 ± 0.12	-0.06 ± 0.15	0.49 ± 0.05
B. wurflenii	1.66	-	-2.51**	-1.70***	-1.03***	-	-4.89***	-1.80	-2.49**	-	65.2	0.94 ± 0.01	0.56 ± 0.11	0.70 ± 0.10	0.38 ± 0.12

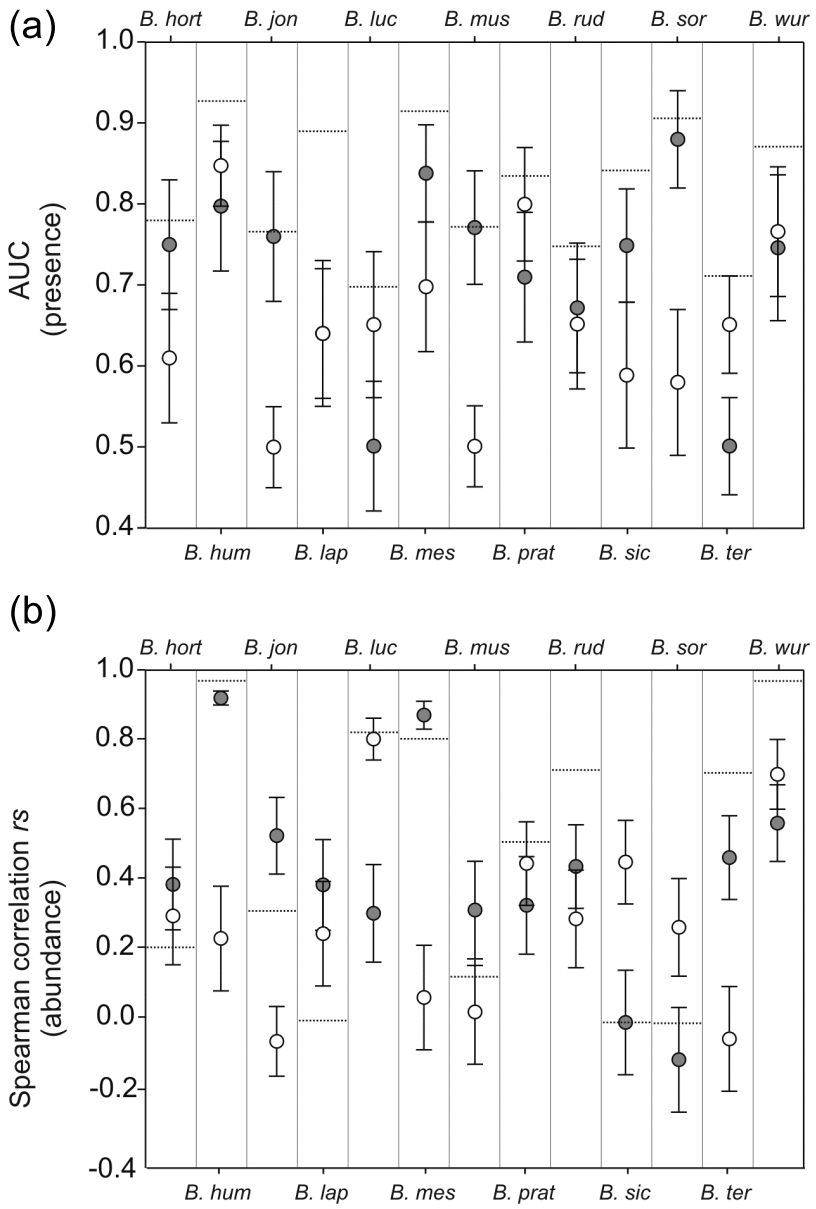

Figure 3.2. Predictability of the probability of bumblebee species' presence (a) and abundance (b) as a function of topoclimatic (grey points) and land-cover (white points) variables. Land-cover variables were extracted from parameters that provided the best fit at each of the seven nested spatial scales (see text for details). Predictability was calculated using AUC (mean ±SE) and Spearman correlation coefficient (r_s; mean ±SE) for bumblebee species' presence and abundance, respectively. Dotted line within each species' area represent predictability (AUC for presence models and Spearman correlation values (r)) of combined models (i.e., including both topoclimatic and land-cover variables).

Congruence of current and past trends

Model structure and relative predictability for both historical (1988-1989) and current surveys (2007-2009) were strongly congruent, suggesting an overall good model performance (Fig. 3.3 and Appendix 3.5 for presence data and Fig. 3.4 and Appendix 3.6 for abundance data, respectively).

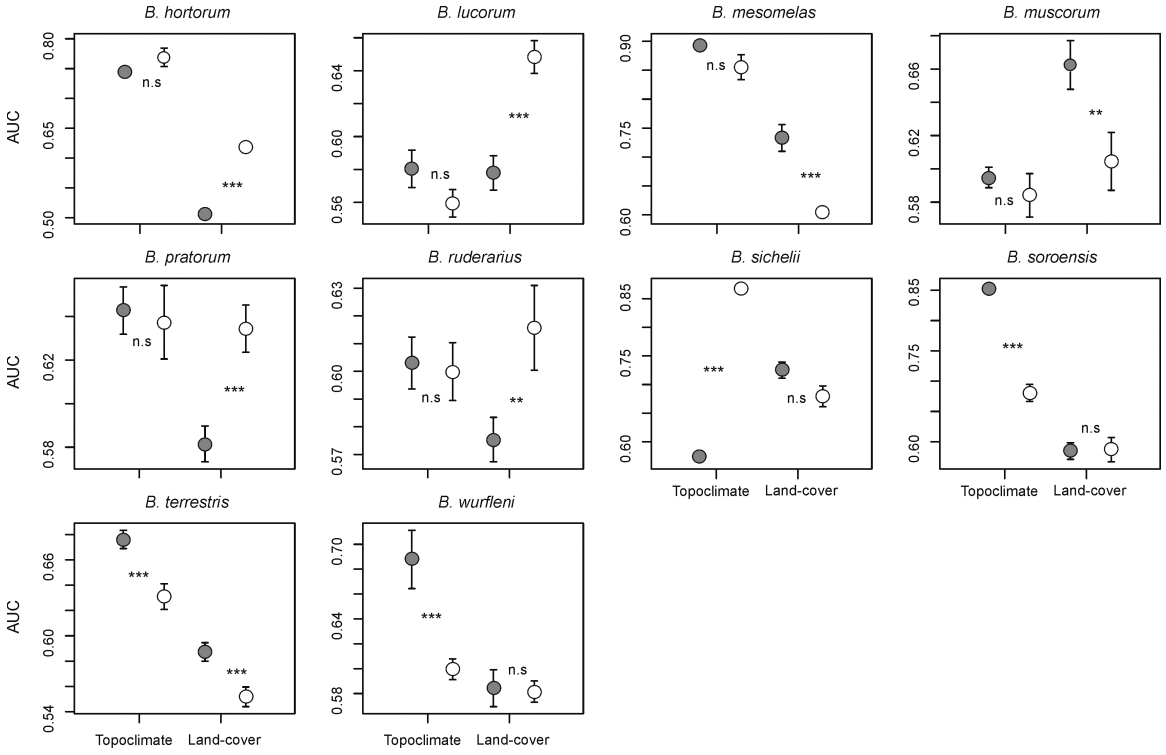

Figure 3.3. Comparison of the predictabilities (AUC, mean ±SE) of bumblebee species' presence between historical (i.e., 1988-1989; grey points) and current surveys (i.e., 2007-2009; white points) as a function of topoclimatic (left pairs within each box) and land-cover variables (right pairs). Only species present in at lest 10 localities in both study periods were included in order to increase model reliability (see main text for details and Appendix 3.5 for a detailed description of model structure). Significance levels (*** P < 0.001, ** P < 0.01, * P < 0.05, n.s. not significant) between each pair of topoclimatic and land-cover variables for individual species are also showed. Note the different scale of the y-axis.

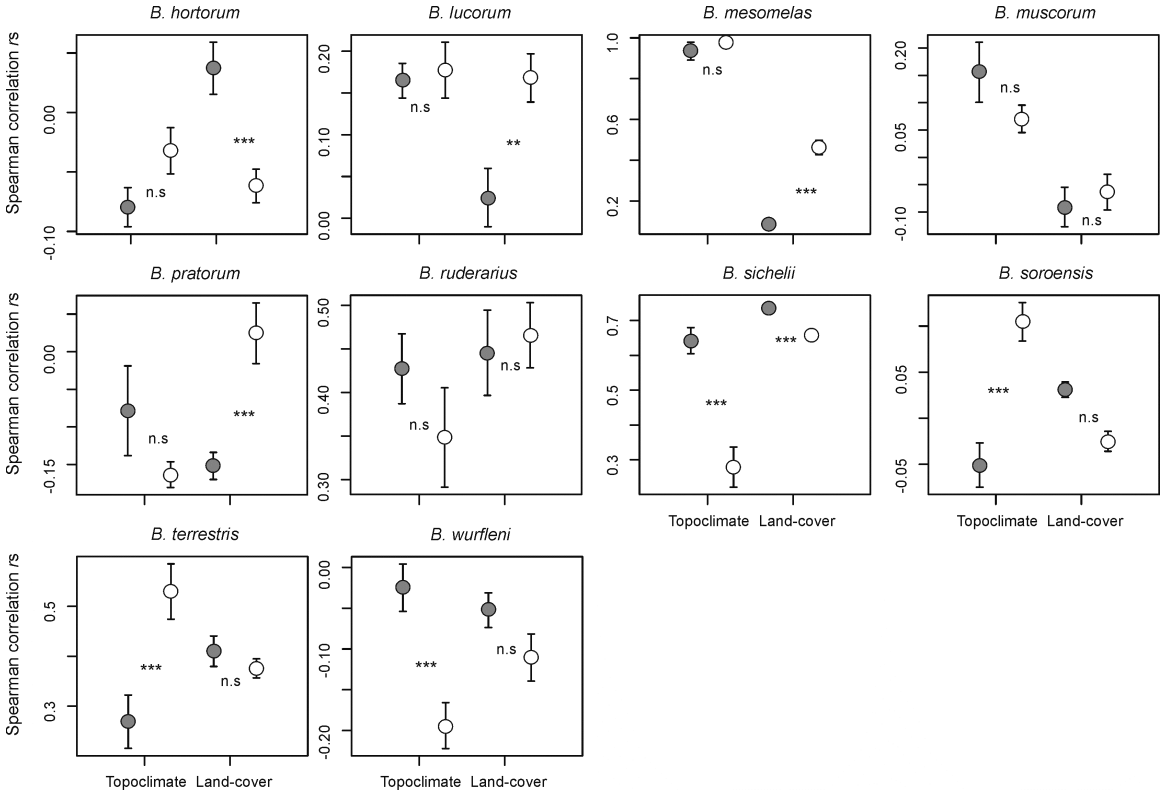

Figure 3.4. Comparison of the predictabilities (Spearman correlation coefficients; mean ±SE) of bumblebee species' abundance between historical (i.e., 1988-1989; grey points) and current surveys (i.e., 2007-2009; white points) as a function of topoclimatic (left pairs within each box) and land-cover variables (right pairs). For further conventions, see Fig. 3.3.

The comparison between the current and historical presence models showed that six species agreed with the predictability of topoclimatic models (Fig. 3.3; Appendix 3.5) and seven species showed strong (i.e., significant) differences for land-cover models (Fig. 3.4; Appendix 3.6). In addition, when significant changes between topoclimatic models of a given species occurred, no changes between land-cover models were observed, and viceversa. This later was true for all species except for *B. terrestris* in which significant changes in both topoclimatic and land-cover models were observed.

Regarding abundance models, the comparison between current and past models performace showed similar patterns to that from species presence for topoclimatic variables. However, the predictability of land-cover models between both time periods changed in four species (*B. muscorum*, *B. ruderarius*, *B. sichelii* and *B. terrestris*), vanishing the general trend found for species' presence (see above) that when significant changes between topoclimatic models of a given species occurred, no changes between land-cover models were observed (Fig. 3.3 and 3.4).

Finally, although topoclimatic models consistently outperformed land-cover models at explaining species' distribution in 1988-1989 and 2007 2009 (70% and 60%, respectively), for species' abundance topoclimatic models showed lower or similar predictability between both study periods (70% and 60%, respectively) (Fig. 3.3 and 3.4; Appendix 3.5 and 3.6). Indeed, relative model performance was not consistent across species between distribution and abundance models (correlation between AUC and r_s, all $r_s > 0.08$, $P < 0.001$, $n = 10$) and cross-validation data (all $r_s > 0.07$, $P < 0.001$, $n = 10$).

DISCUSSION

Here, we investigated the contribution of topoclimate and land-cover to predict the distribution and abundance of bumblebee species in a mountain region. Thanks to two datasets separated by a time span of 20 years, we examined the reliability of our model predictions as well as the potential role of topoclimate and land cover for forecasting variation in species responses in the face of regional climate warming. We found that land cover, but especially topoclimatic variables, explained an important amount of the variability in species' distribution and abundance. The congruence between models developed using past and current data sources supported the consistency of our results. Whereas some species had no changes in model predictability for topoclimatic variables and significant changes for land-cover variables, the opposite was true for the remaining species,

suggesting species-specific responses of topoclimatic and land-cover variables, and in turn, species-based vulnerability to changes in environmental conditions.

Topoclimatic vs land cover effects

Previous studies have suggested that topoclimate (by imposing range limits either through direct constraints or, indirectly, by mediating biotic interactions such as competition) plays a prominent role on species' distribution especially at broad spatial scales (e.g., Jetz and Rahbek 2002, Hawkins et al. 2003, Heikkinen et al. 2007). In contrast, land-cover variables (by determining food-resource availability and habitat structure and composition within localities) would shape species' distribution patterns at fine spatial scales (Araújo et al. 2005). Our findings, however, illustrate that fine-scale bumblebee distribution was mainly governed by topoclimate, even when using different data sources (1988-1989 versus 2007-2009) and different response variables (presence/absence versus abundance). Our results were in line with other works investigating the determinants of species' distribution and abundance of insect species in mountain areas (Stefanescu et al. 2004, 2011, Gutiérrez-Illán et al. 2010). Indeed, the results emerging from our work highlight the idiosincrasy of mountain systems, where topography commonly modifies macroclimatic patterns to produce strong microclimatic gradients along which species are distributed that are even able to obscure the effect of the local habitat features. Such a "scale-dependent problem" highlights avenues for future research within this framework such as investigating which are (and how they interact) the actual environmental determinants of species distribution in mountain systems and how species-specific traits related to them (e.g., the degree of climatic and habitat specialization) might influence population dynamics and community structure (Angert et al. 2011). Accordingly, fine-grained studies coupled with relevant natural history and individual species' biology would provide a more robust framework for predicting the response to global change drivers such as climate warming as well as forecasting variations across species.

Nevertheless, not only topoclimatic but also land-cover variables were included in both distribution and abundance models. Indeed, combined models (i.e., those including both topoclimatic and land-cover variables) consistently gave the best model performance (Fig. 3.2). For example, land cover variables outperformed topoclimatic variables for *B. humilis*, *B. lucorum* and *B. pratorum*. Indeed, combined models (i.e., those including both topoclimatic and land cover variables) consistently gave the best model performance in almost all bumblebee species. Land-cover effects were scale-dependent and species-specific as bumblebees showed individualistic responses to land-cover variables at different spatial scales. Therefore, far from trivial, our findings suggest that land-cover variables also determine fine-scale distribution and abundance patterns of bumblebees (see Appendix 3.2 and Appendix 3.3). Although scale-dependence in landscape context effects has been previously showed to influence spatial distribution patterns of insects species such as bees (e.g., Steffan-Dewenter et al. 2001), these effects have not always been found for bumblebee species (Steffan-Dewenter et al. 2002; but see Westphal et al. 2006). Likely explanations include that previous studies investigated landscape-context effects on bumblebee distribution at community level, thus neglecting the potential idiosyncrasy of each individual species' realized response. Furthermore, unlike plains and most flat landscapes where the study of landscape-context effects on insects have been commonly carried out, mountain ecosystems show strong elevational gradients in landscape structure which might determine vegetation heterogeneity high enough to drive scale-dependent effects. In any case, our findings suggest that the land-cover variables we use in the present study were suitable to test landscape effects (and its scale-dependence) on spatial distribution patterns of these insect species. This was true even when performing simplifications of the landscape structure by integrating several land-cover types into single groups (see Steffan-Dewenter et al. 2002 for similar conclusions). This is likely related to the fact that, unlike other insects such as butterflies, most bumblebees are generalists with no significant specialization on particular resource-plant species and host plants for larval development. Thus, we did not expect significant relationships between bumblebees and any land-cover type and/or plant species.

Past and current trends: determining species' vulnerability to global change

In a previous study, we investigated changes in spatial distribution patterns of bumblebees in the Cantabrian Range over the last two decades (see Chapter 1). We found that changes in the lower boundary of most species as well as mean population altitude parallel the 160 m rise in isotherms due to the regional warming of 0.8°C (i.e., temperature lapse rate in our study region is by -0.5°C 100 m^{-1}; Mateo 1970). Furthermore, significant changes in landscape structure of sampled localities between the historical (1988-1989) to the current (2007-2009) study periods looked negligible. We therefore suggested that elevational range shifts were potentially driven by changing climatic conditions (especially rising temperatures) rather than by changes in habitat structure. Yet, this hypothesis was undertaken and explicit relationships between species distribution and patterns of climate and land-cover change were not addressed. The dominant role of topoclimate over land-cover emerged from our results, and thus provides additional support for the hypothesis of climate-driven distribution changes over the last decades. This finding agrees to that previously found for butterfly species in the Sierra de Guadarrama (central Spain) where climate was a key determinant of species richness, and climate warming one of the most relevant driving forces of change in community composition (Stefanescu et al. 2003, 2004, Wilson et al. 2007, Gutiérrez-Illán et al. 2010).

The relative role of climate warming at determining changes in species' distribution and abundance over the last two decades are illustrated in Fig. 3.3 and Fig. 3.4, respectively. As the only significant change in the regional landscape-scenario between 1988-1989 and 2007-2009 is climate warming, no significant changes in model predictability for topoclimatic variables between both study periods would suggest that species are tracking suitable climatic conditions (especially temperature). On the other hand, because we analyzed the same set of localities for both study periods, significant changes in model predictability for land-cover variables would suggest that species have changed the identity of localities at which they occurred from one period to another. This mean that six

species (*B. hortorum*, *B. lucorum*, *B. mesomelas*, *B. muscorum*, *B. pratorum* and *B. ruderarius*) consistently tracked climate warming via range shifts irrespective to the landscape structure of target locations. On the contrary, three species (*B. sichelii*, *B. soroeensis* and *B. wurflenii*) exhibited an opposite trend (i.e., significant changes in model predictability for topoclimatic but no changes for land-cover variables, specially at large scales), suggesting the prevalence effect on landscape structure of sampling localities. One exception was *B. terrestris*, a highly generalist species which showed significant changes in model predictability for topoclimatic as well as land-cover variables (Williams et al. 2009). We found, however, no relationship between the degree of dependence on topoclimatic and land-cover variables, and the extent of each species' response. This is likely related to the fact that although topoclimatic performed better than land-cover variables in both presence and abundance models, in general, combined models obtained higher model performance than purely topoclimatic and land-cover models. Overall, the considerable species-specific variation in the relative role of topoclimatic and land-cover variables suggest that individualistic traits might interact to modulate each species' realized response to climate warming. Therefore, understanding the capacity of the different species to track rapid climate change via range shifts seem crucial both to understand the biological effects of, and responses to, climate change, and open new areas for future research.

Conclusions

We found that land cover, but especially climate, play a key role in determining fine-scale bumblebee species' distribution and abundance in the mountain region of the Cantabrian Range (north-west Spain). Our results therefore suggest that using topoclimate could provide a highly informative landscape scenario for predicting species' distribution patterns in mountain systems as well as project future distributions under various scenarios of environmental change. In addition, our findings suggest that species' abilities to track climate change via range shifts were idiosyncratic, making it difficult to disentangle the effect of land-cover and

climate on changes in individual species distribution. Accordingly, our findings highlighted the need for species-specific approaches to develop more predictive frameworks for individualistic vulnerability assessments, and therefore, biologically meaningful approaches for conservation management planning.

4

Variation in trophic niche width and overlap in bumblebee assemblages along an elevational gradient: importance of species richness and life history traits

INTRODUCTION

Species' abundance and composition in any assemblage is determined by interspecific competition for resources and physiological constraints associated with environmental factors (Pulliam 1975, Hopf et al. 1993, Tilman 2004). These two processes (i.e., competition and habitat selection reflecting ecological tolerances to abiotic factors) can have important consequences on the distribution of functional traits of coexisting species (Hutchinson 1959, Cornwell et al. 2006). For instance, smaller ecological niches might be expected in species-rich assemblages, where interspecific competition should be stronger (Pulliam 2000). On the contrary, the niche variation hypothesis (NVH; Van Valen 1965, MacArthur et al. 1972, Costa el al. 2008) predicts niche expansion in species-poor assemblages due to weaker interspecific competition. This niche expansion usually occurs via increased variation among individuals rather than expansion of individual niches (Bolnick et al. 2007). On the other hand, variation in species richness is a widespread phenomenon across latitude and elevation gradients (Rahbek 1995, Nogués-Bravo et al. 2008). Thus, the quantification of the niche width of coexisting species along environmental gradients of species richness, such as an elevational gradient, should reveal the relative impact of these processes (i.e., competition and abiotic factors) in shaping ecological communities (e.g., Laughlin et al. 2012).

Bumblebees (*Bombus* spp.) are among the most important pollinators in cold and temperate regions (Bingham and Orthner 1998, Ricketts et al. 2008). There is now ample evidence that bumblebee species have declined and, given their importance as pollinators, there is strong interest in their conservation (e.g., Kosior et al. 2007, Cameron et al. 2011). Bumblebees are social insects, feeding exclusively on nectar and pollen from the plants they visit throughout their lives. Because a large number of species usually coexist in small locations and it is relatively easy to observe most species foraging at the same time, they are particularly well suited to investigate community structure (e.g., Hanski 1982, Pyke 1982, Ranta et al. 1984). Besides, bumblebees are a rather homogeneous

group except for marked differences in their tongue length, which determines the preferred range of flower types they visit and it has been suggested to determine species composition in bumblebee assemblages (Inouye 1978, Pyke 1982, Ranta et al. 1984). Although it is widely assumed that most bumblebee species are generalists, it has been suggested that long-tongued species have more specialized diets (Heinrich 1976, Goulson and Darvill 2004, Goulson et al. 2005).

However, assessing the trophic niche of bumblebees could have temporal and spatial limitations given that data should be collected under comparable conditions and bumblebees should be exposed to the same array of choices (Williams 2005). Thus, comparing niche width between common and rare species can bias the results obtained in analyses of flower visits; rare species would yield lower number of visits, and thus may appear to visit a narrower range of plants (Williams 2005). Furthermore, observation of the number and species of flowers visited and analysis of pollen loads carried by bumblebees may biased the results because they represent single feeding events ('snapshots') rather than the long-term resource use of individuals (Goulson and Darvill 2004, Goulson et al. 2005, Kleijn and Raemakers 2008). This is especially important given that bumblebee workers are known to change floral preferences in response to abundance in variation of floral resources (Heinrich 1976, 1979).

Stable isotopes analysis can be a robust alternative to estimate trophic niche width because it provides a time- and space-integrated record of resource assimilation (Fry et al. 1978, Dalerum and Angerbjörn 2005). Because the stable isotopes ratios of nitrogen ($^{15}N/^{14}N$, expressed as $\delta^{15}N$) and carbon ($^{13}C/^{12}C$, $\delta\,^{13}C$) on the tissues of the consumers reflect the stable isotopes of their diet, the relative position of organisms in $\delta\,^{13}C$ - $\delta^{15}N$ bi-plot space can be used as a representation of the population trophic niche (Newsome et al. 2007, Wolf et al. 2009). Moreover, quantitative measures such as the area of niche space occupied and the degree of isotopic niche overlap among populations can be used to compare multiple systems across environmental gradients (Layman et al. 2007*a*, Jackson et al. 2011).

Here, we analyzed trophic niche width and niche overlap by means of stable isotope analysis in bumblebee assemblages across an elevational gradient in the Cantabrian Range (NW Spain). The study area has a great diversity of bumblebee species (up to 24 species have been described in the region, excluding parasitic bumblebees of sub-genus *Psithyrus*) and previous studies found that bumblebee species richness peaks at intermediate middle elevations (Obeso 1992). The strong elevational gradient found in the region (0 to 2650 m a.s.l) provides a natural experiment to compare bumblebee assemblages with different species richness. The main goal of this study is to determine whether bumblebee trophic niche varied along the elevational gradient. Specifically, we tested whether: (1) niche breadth is inversely related to species richness in bumblebee assemblages; (2) this niche expansion is due to increased variation among individuals rather than increased individual niche breadth, and (3) tongue length, an important functional trait in bumblebees, could explain differences in niche width and niche overlap among coexisting species.

MATERIAL AND METHODS

Study sites

We conducted this study during the spring and summer of 2010 in the Cantabrian Range (provinces of Asturias and León, NW Spain, Fig. 4.1). We sampled bumblebee assemblages in 6 localities along an elevational gradient. We selected sampling sites based on previous surveys of bumblebee presence and habitat suitability (Table 4.1). Minimum distance between two adjacent sites was 6.76 km (Fig 3.1). Because most bumblebee species have a foraging range < 500 m (although up to 2 km for *B. terrestris*), bumblebee faunas of different fields are expected to be independent (Kreyer et al. 2004, Knigth et al. 2005, Hagen et al. 2011).

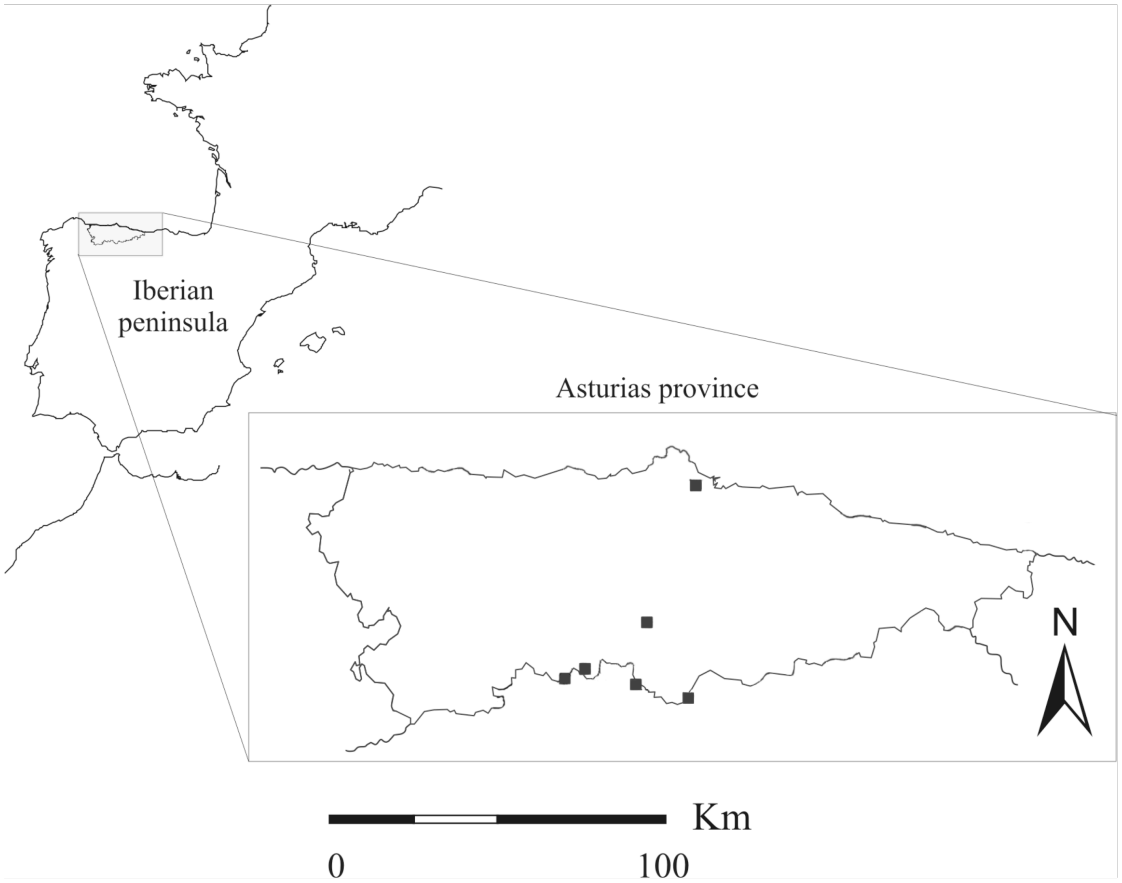

Figure 1. Location of the study sites in the Cantabrian Range (NW Spain).

Table 1. Summary of sample sites characteristics.

Site	Elevation (m a.s.l.)	Location	Dominant habitat type
Prendes	73	43 33 07.3 N, 05 45 44.8 O	Mowed meadow
Brañilín	1418	42 59 13.5 N, 05 45 41.8 O	Pastures and heathlands
Valle	1470	43 03 00.8 N, 06 08 46.3 O	Mowed meadow
El Puerto	1565	43 01 20.8 N, 06 13 09.4 O	Heathlands
Gamoniteiru	1712	43 11 09.9 N, 05 55 14.3 O	Pastures
Peña Ubiña	2218	43 00 48.0 N, 05 57 25.8 O	Pastures

Bumblebee and pollen collection

At each site, we caught bumblebee workers from mid May to late August 2010, in order to cover the maximum flower blooming and thus all the bumblebee species present at the study sites. We limited our analysis to bumblebee workers to minimize the variability associated to potential differences between castes, which have been found in other social insects (Blüthgen et al. 2003). We conducted captures between 09.00 h and 17.00 h in warm and dry weather, when bees are more active. We searched the area thoroughly for target species during several days in order to achieve enough workers of each species. However, samples sizes were inevitably larger for the most abundant species (see Table 4.2 for abundance and characteristics of bumblebee species present in this study).

Table 4.2. Abundance and tongue length (mean ± SE) of *Bombus* species present in this study.

	Site †						Tongue length (mm) ‡
Species	**Prendes**	**Brañilín**	**Valle**	**El Puerto**	**Gam.**	**P. Ubiña**	
B. hortorum	2	6	---	2	2	1	11.08 ± 0.65 (13)
B. humilis	**13**	**18**	---	**24**	1	---	7.99 ± 0.19 (50)
B. jonellus	---	**14**	4	2		---	6.08 ± 0.22 (20)
B. lapidarius	---	5	2	4	**25**	2	6.03 ± 0.11 (36)
B. lucorum	---	**20**	2	8	1	---	6.0 ± 0.18 (31)
B. mesomelas	---	---	1	**19**	3	**25**	7.58 ± 0.11 (40)
B. mucidus	---	---	7	---	1	---	6.09 ± 0.34 (7)
B. muscorum	---	1	4	**16**	---	---	7.26 ± 0.11 (21)
B. pascuorum	**11**	---	---	---	---	---	9.84 ± 0.42 (11)
B. pratorum	---	6	---	4	---	---	6.44 ± 0.21 (9)
B. ruderarius	---	3	9	**25**	---	2	7.77 ± 0.15 (36)
B. sichelii	---	2	**10**	2	**24**	**25**	5.60 ± 0.09 (61)
B. soroeensis	---	**25**	**16**	6	8	**25**	6.29 ± 0.08 (78)
B. sylvarum	**15**	---	---	6	---	---	8.00 ± 0.29 (20)
B. terrestris	**25**	7	---	1	3	1	8.23 ± 0.21 (36)
B. wurflenii	---	**19**	3	**15**	7	6	7.16 ± 0.14 (43)

† In bold are shown the species for which SEA_c was calculated.
‡ The number of individuals used to estimate tongue length are given in parentheses.

We recorded the flower species that the bee was visiting, and whether they carried pollen or not. When bumblebees were carrying pollen in their hind legs, we carefully removed the pollen loads using forceps and transferred them to a small container. We cleaned the forceps between the handling of different specimens to avoid contamination. We identified all bumblebees captured to species level under a stereomicroscope. After collection, bumblebee workers and pollen samples were stored frozen (- 20°C) prior to stable isotope analysis.

Morphological measurements

We measured tongue lengths of each worker as described in Ranta et al. (1984). Tongue length is defined as the total length of prementum and glossa, following Prys-Jones and Corbet (1991). Tongues were scanned and length of the tongue was measured to the nearest 0.01 mm in Image J (Schneider et al. 2012; Table 4.2). We also measured thorax length (a reliable measure of body size for bumblebee workers, Goulson and Sparrow 2009) using a magnifying glass equipped with an ocular micrometer, and a caliper (Leika M125). We calculated analysis of variance (ANOVA)-based repeatabilities (R_A) of morphological measurements (tongue length: $R_A = 0.99$, $P < 0.001$, $n = 34$ repeated measures; thorax width: $R_A = 0.96$, $P < 0.001$, $n = 35$; Nakagawa and Schielzeth 2010).

Stable isotope analysis

We measured $\delta^{13}C$ and $\delta^{15}N$ on bumblebee thorax to determine trophic niche width and niche overlap between bumblebee species. We only included thorax tissue to eliminate the effect of undigested food on isotopic measurements. The thorax of the bumblebees, which contains the flight muscles, is basically composed of insect cuticle (i.e. chitin) and muscle tissue. Prior to stable isotope analysis, we cleaned samples to remove pollen and parasites. Each sample was ground to a fine, homogeneous powder. Previous assays on bumblebee thorax suggested that lipid

content might have a significant effect on $\delta^{13}C$ estimates of bumblebee samples, whereas lipid extraction had no effect on $\delta^{15}N$ (data not shown). Thus, we removed lipids with a 1:1 chloroform-methanol solution for 30 min (Van Handel 1985), oven-dried (40-50°C) for 48 h and kept dry until analysis. We used the same procedure with pollen samples. We packed subsamples of 1 mg (± 0.2) into 5x8 mm tin capsules for $\delta^{13}C$ and $\delta^{15}N$ analyses, which were done using a continuous-flow isotope ratio mass spectrometer at the UC Davis Stable Isotope Facility (USA). Experimental precision was estimated as the standard deviation of replicates, and was 0.09 ‰ for $\delta^{13}C$ and 0.18 ‰ for $\delta^{15}N$.

Stable isotope ratios are expressed in δ notation, as part per thousand deviation from standard material, PeeDee belemnite limestone for $\delta^{13}C$, and atmospheric nitrogen for $\delta^{15}N$, according to the equation:

$$\delta X = [(R_{\text{sample}} - R_{\text{standard}}) - 1] \times 1000$$

where X is for ^{15}N or ^{13}C and R is the corresponding ratio ^{15}N:^{14}N or ^{13}C:^{12}C.

We use the pollen loads from the hind legs of bumblebee workers to obtain baseline signatures for each site (Post 2002). The $\delta^{13}C$ and $\delta^{15}N$ baselines were obtained by averaging values of pollen loads from at least 8 different workers (see Appendix 4.1 for details).

We calculate the trophic fractionation factor (Δ) as the difference between the isotopic baseline of pollen samples and bumblebee samples (Post 2002). We then standardize $\delta^{13}C$ and $\delta^{15}N$ values of bumblebee workers prior to calculations in order to allow cross-ecosystem comparisons. We normalized $\delta^{13}C$ and $\delta^{15}N$ values by calculating their Z scores: $Z = (X - \bar{X}) / s$, where X is the value of the variable for each sample, $\bar{X}$ is the mean value of the isotopic baseline at each study site and s is its SD (Moreno et al. 2006, Hoeinghaus and Zeug 2008).

Trophic niche width of bumblebee species

To calculate trophic niche width of bumblebee species, we used standard ellipses areas (SEA_c) corrected by sample size metric comparable to univariate standard deviation (Jackson et al. 2011). By using individuals plotted in a $\delta^{13}C$ - $\delta^{15}N$ space as measurements units, SEA_c can be used as a measure of the mean core population isotopic niche. Contrary to other community-level metrics based on stable isotope measurements (Layman et al. 2007*a*), SEA_c are unbiased with respect to sample size and allows robust comparison among data sets (Jackson et al. 2011). However, a minimal sample size of 10 is recommended, and thus we only calculate SEA_c for species with $n \geq 10$ (Jackson et al. 2011). Accordingly, 21 populations from 13 species meet the assumptions (Table 4.2, Fig. 4.2). Thus, we were able to calculate niche overlap for 29 species pairs. We measured niche overlap between coexisting species by quantifying the percentage of area that were shared by both species' SEA_c.

To estimate the trophic variability within each population, the mean distance to centroid (CD) was used as a measure of trophic diversity (Layman et al. 2007*a*). CD was calculated as the Euclidean distance of each individual to the $\delta^{15}N$ – $\delta^{13}C$ centroid of its population. Then we calculated the coefficient of variation of the distances from each individual to all its neighbors in the isotopic space (CVND), which gives a measure of the clustering of values and trophic redundancy (Quevedo et al. 2009).

Data analysis

We calculated SEA_c and niche overlap following methods from Jackson et al. (2011) and the package "siar" (Parnell and Jackson 2011), an extension to R statistical environment (R Development Core Team 2012), which we used for all the statistical analysis.

We used one-way ANOVA and post hoc Tukey tests to assess differences on trophic niche width (SEA_c), CD, CVND and % overlap among sites with different species richness. Response variables did not deviate from the normal distribution in any case (Kolmogorov-Smirnov tests, $P > 0.05$). Before performing ANOVA tests, SEA_c values were transformed by means of a Box-Cox transformation ($y = (x^{\lambda} - 1)/\lambda$, $\lambda = 0.14$) to attain homogeneity of variances (Box and Cox 1964).

Additionally, we used mixed-effects models (LMM) to evaluate the relationship between (1) % overlap difference on tongue length between species pairs, and (2) SEA_c and tongue length or body size. In these LMM, we regarded difference in tongue length between species pairs, tongue length and thorax width (body size) as fixed effects, and site (elevation) and/or species identity as random effects (see Appendix 4.2 and 4.3). We obtained model fitting and estimates with linear mixed-effects (lmer) package *lme4* (Bates et al. 2011), using REML (restricted log-likelihood) method. We ran Markov Chain Monte Carlo (MCMC) sample with 10 000 simulations using the function "pvals.fnc" from the *languageR* package (Baayen 2011) to generate *P* values for LMM. Throughout the paper, we present MCMC-estimated *p*-values that are considered significant at the $\alpha=0.05$ level.

RESULTS

Plant visits and pollen baseline

Bumblebee species visited 23 plant species from 12 families in the study sites. The proportion of visits in the different plant families was 33.7% Ericaceae, 29.0% Apiaceae, 22.04% Asteraceae, 6.01% Orobanchaceae, 4.55% Lamiaceae, 2.9% Fabaceae and 1.8% others. In bumblebees, all samples except one were more enriched in $\delta^{13}C$ (mean difference to pollen sample ± SD, 1.32 ± 0.6‰) and $\delta^{15}N$ (1.46 ± 1.45‰) than their pollen baseline (Appendix 4.1). These values fall within the range reported for other invertebrates (Blüthgen et al. 2003, Vanderklift and Ponsard 2003).

Variability in trophic niche space in bumblebee assemblages

Bumblebees showed a smaller total niche space (SEA_c) in species- rich assemblages relative to species- poor assemblages at both species and assemblage level (Table 4.3 and 4.4). At the species level, the difference ranged from 97.8% in the case of *B. mesomelas* to 50% in *B. soroeensis* (Table 4.3). The reduction of SEA_c was due to a significant decrease in CVND in the case of *B. humilis*, *B. soroeensis* and *B. wurflenii*, which suggest increased variation among individuals in depauperate assemblages (Table 4.4). Total niche space of bumblebee species was not determined by tongue length (t = 0.436, P_{MCMC} = 0.48, Markov Chain Monte Carlo [MCMC]; Appendix 4.2) or thorax width (t = 0.049, P_{MCMC} = 0.174; Appendix 4.2).

At the assemblage level, there were significant differences on total niche space (SEA_c) among sites with different species richness (Table 4.4). The mean trophic niche space (SEA_c) was smaller in species-rich assemblages (Table 4.4, Fig. 4.2). Additionally, CD and CVND showed larger values in species-poor assemblages, which suggest greater among-individual variation in more generalized populations (Table 4.4). Percentage niche overlap between species pairs was greater in species-rich assemblages (Table 4.4, Fig. 4.2). The degree of isotopic niche overlap in bumblebee species ranged from 0% (no overlap) between *B. soroeensis* and *B. jonellus* (Fig 4.2d) to 88.74% overlap between *B. ruderarius* and *B. muscorum* (Fig 4.2f). There was a significant negative relationship between niche overlap and difference in tongue length between species pairs (t = 0.026, P_{MCMC} = 0.026, see Appendix 4.3) suggesting a similar resource use among coexisting species of similar tongue length.

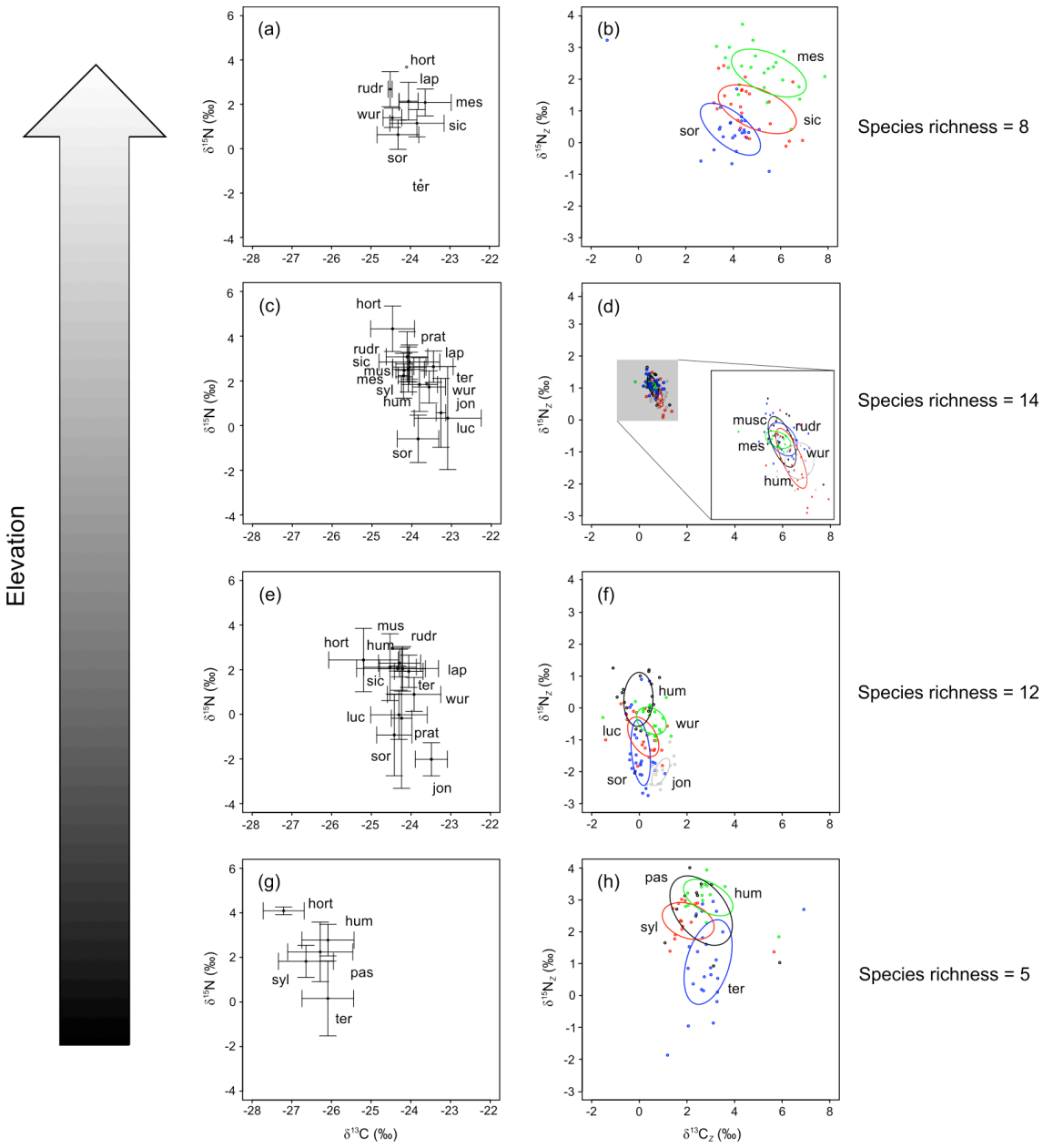

Figure 4.2. Stable isotope bi-plots (mean ± SD) for each site (a-b, Peña Ubiña; c-d, El Puerto; e-f, Brañilín; g-h, Prendes), illustrating the isotope niche and niche overlap for bumblebee species. The lines enclose the standard ellipse area (SEA_c) for each species. $\delta^{13}C_Z$ and $\delta^{15}N_Z$ correspond to standardized *Z* scores of isotopic values. Note: hort, *Bombus hortorum*; hum, *B. humilis*; jon, *B. jonellus*; lap, *B. lapidarius*; luc, *B. lucorum*; mes, *B. mesomelas*; muc, *B. mucidus*; mus, *B. muscorum*; pas, *B. pascuorum*; prat, *B. pratorum*; rudr, *B. ruderarius*; sic, *B. sichelii*; sor, *B. soroensis*; syl, *B. sylvarum*; ter, *B. terrestris*; wur, *B. wurflenii*.

Table 4.3. Population metrics of various bumblebee species along the elevation gradient, and comparisons among sites with different species richness (mean ± SD).

		Species								
		B. humilis			***B. mesomelas***			***B. sichelii***		
Site	**Species richness**	**SEA_c**	**CD**	**CVND**	**SEA_c**	**CD**	**CVND**	**SEA_c**	**CD**	**CVND**
Prendes	5	1.63	0.79 ± 0.78 a	0.81 ± 0.29 a	---	---	---	---	---	---
Brañilín	12	1.63	0.89 ± 0.43 a	0.49 ± 0.09 b	---	---	---	---	---	---
Valle	10	---	---	---	---	---	---	1.08	0.71± 0.39 a	0.51 ± 0.10
El Puerto	14	0.23	0.42 ± 0.22 b	0.54 ± 0.10 b	0.07	0.18 ± 0.14	0.61 ± 0.16	---	---	---
Gamoniteiru	10	---	---	---	---	---	---	1.54	0.76 ± 0.67 a	0.77 ± 0.24
Peña Ubiña	8	---	---	---	3.22	1.40 ± 0.94	0.55± 0.13	3.42	1.46 ± 0.97 b	0.64 ± 0.21
Total change†		-85.9 %			97.8 %			68.4 %		
Statistics			$F_{2,52}$ = 5.90; P = 0.005	$F_{2,52}$ = 16.16; P < 0.001		t = -6.39; P < 0.001	t = 1.28; P = 0.21		$F_{2,56}$ = 6.04; P = 0.004	$F_{2,56}$ = 6.11; P = 0.004

Notes: SEA_c represents standard ellipses areas; CD shows the mean distance of each individual to the isotopic centroid of its population; CVND, coefficient of variation of distances from each individual to all its neighbors in the isotopic space. *F* values correspond to one-way ANOVA, and *t* values correspond to Student's *t* test. Different letters indicate significant differences among groups after Tukey tests ($P < 0.05$).

† Change in total niche space (SEA_c) from lower to higher elevations.

Table 4.3. Continued.

		Species					
		B. soroensis			*B. wurflenii*		
Site	**Species richness**	**SEA_c**	**CD**	**CVND**	**SEA_c**	**CD**	**CVND**
Prendes	5	---	---	---	---	---	---
Brañilín	12	1.25	0.89 ± 0.58	0.63 ± 0.18 a	0.8	0.59 ± 0.42	0.61 ± 0.17
Valle	10	1.45	0.98 ± 0.98	0.84 ± 0.29 b	---	---	---
El Puerto	14	---	---	---	0.19	0.29 ± 0.42	0.43 ± 0.08
Gamoniteiru	10	---	---	---	---	---	---
Peña Ubiña	8	2.5	0.94 ± 1.13	0.97 ± 0.31 b	---	---	---
Total change†		50 %			-76.25 %		
Statistics			$F_{2,63}$ = 0.04; P = 0.96	$F_{2,63}$ = 10.49; P < 0.001		t = 2.87; P = 0.009	t = 4.22; P < 0.001

Notes: SEA_c represents standard ellipses areas; CD shows the mean distance of each individual to the isotopic centroid of its population; CVND, coefficient of variation of distances from each individual to all its neighbors in the isotopic space. F values correspond to one-way ANOVA, and t values correspond to Student's t test. Different letters indicate significant differences among groups after Tukey tests (P < 0.05).

† Change in total niche space (SEA_c) from lower to higher elevations.

Table 4.4. Comparisons of trophic niche metrics (mean ± SD) for $\delta^{13}C$ and $\delta^{15}N$ among sites.

Site	Sample size	Species richness	SEA_c†	% Overlap	CD†	CVND†
Prendes	66	5	2.86 ± 1.28 a	10.37 ± 10.80 ab	1.03 ± 0.28 ac	0.72 ± 0.27 a
Brañilín	126	12	1.04 ± 0.47 b	5.46 ± 7.92 b	0.72 ± 0.19 a	0.56 ± 0.15 b
El Puerto	134	14	0.17 ± 0.06 c	19.98 ± 12.05 a	0.30 ± 0.08 b	0.52 ± 0.13 b
Peña Ubiña	87	8	3.06 ± 0.47 a	1.38 ± 2.13 b	1.27 ± 0.29 c	0.72 ± 0.29 a
Statistics			$F_{3,13} = 36.56$, $P < 0.001$	$F_{3,25} = 4.71$, $P = 0.009$	$F_{3,13} = 16.22$, $P < 0.001$	$F_{3,330} = 20.87$, $P < 0.001$

Notes: SEA_c represents standard ellipses areas; % overlap; CD, distance of each individual to the isotopic centroid of its subpopulation; CVND, coefficient of variation of distances from each individual to its neighbors in the isotopic space. SEA_c and % overlap were only calculated where a sample size of $n > 10$ was obtained. Therefore, mean SEA_c and % overlap were not calculated for the Valle and Gamoniteiru sites because only two species at each site meet the assumptions. *F* values correspond to one-way ANOVAs.

† Different letters indicate significant differences among groups after Tukey tests ($P < 0.05$).

DISCUSSION

In this study, we show that species richness determines total niche space in bumblebee assemblages along an elevational gradient. We found that bumblebee species showed narrower (more specialized) trophic niches and a greater degree of niche overlap between species pairs in species-rich communities, suggesting a more specialized diet and stronger interspecific competition in those communities. Increased population niche width in less rich assemblages seemed consequence of increased among-individual niche variation. Furthermore, there was a negative relationship between niche overlap and the difference in tongue length between species pairs, which suggest a similar resource use among species of similar tongue lengths. Finally, the trophic niche width of bumblebee species was not

determined by morphological traits (tongue length, body size), but varied significantly along the elevational gradient.

The niche variation hypothesis (NVH) predicts that release from interspecific competition will promote niche expansion in species- poor assemblages. In addition to greater trophic niche in species-poor assemblages, larger values of distances to the isotopic centroid (CD) and CVND indicated higher trophic diversity within the subpopulation, i.e., bumblebees showed greater variability in resource use in species-poor habitats. Therefore, the results suggests that larger population niche breadth is achieved by an increase of among-individual variation rather than an increase of within individual variation, which is in concordance with the conclusions of Bolnick et al. (2007) and Costa et al. (2008) and thus support the NVH (Van Valen 1965, Soulé and Stewart 1970, Bolnick et al. 2007).

Variability on the ecological niche of a species may depend both on the environmental conditions and interference with potential competitors (Pulliam 2000). Inouye (1978) suggested that competitive release in bumblebee species led to rapid expansion in the pattern of resource utilization. Recent findings also support that the relative abundance of host plant and composition of flowers might substantially affect choice behavior and resource use in bumblebees (Goulson et al. 2008*a*, Kleijn and Raemakers 2008). Thus, there is ample evidence that the flexibility in the trophic behaviour of bumblebee species is a widespread phenomenon even at large geographical scales (Kleijn and Raemakers 2008).

In this study, the trophic niche of bumblebee species varies similarly with species richness along the elevational gradient found in the study region. More specifically, both trophic niche space and species richness showed a minimum and maximum, respectively, at medium elevations. Although species-richness is thought to decrease towards higher elevations and thus mirror the well-known latitudinal diversity gradient, the pattern in species-richness is usually hump-shaped when the entire elevational gradient is surveyed (Rahbek et al. 2005, Nogués-Bravo et al. 2008). This hump-shaped pattern in diversity is caused by the

global reduction in natural lowland habitats as a result of increased anthropogenic impact, and the decrease in habitable area and energy limitation towards higher elevations (Hawkins et al. 2003, Nogués-Bravo et al. 2008). A third mechanism is likely to explain the pattern of bumblebee richness in the Cantabrian Range. The area constitutes the southern limit of distribution of many species, which are found in the study region above medium elevations; whereas widespread species at lower elevations are those that have more southern distributions (Rasmont 1988, Rasmont and Iserbyt 2010). The distributions of these species overlap at medium elevations resulting in the observed peak in bumblebee diversity.

Consistent with this, mountain species such as *B. mesomelas*, *B. sichelii* and *B. soroeensis* showed a maximum in their trophic niche width at higher elevations. *B. mesomelas* and *B. sichelii* are only found in southern European mountains at higher elevations (Iserbyt 2009). Similarly, *B soroensis* is a widespread species in West-Palaearctic Europe which is restricted to higher elevations in southern locations (e.g., Rasmont 1988, Iserbyt 2009). On the other hand, *B. humilis*, which is at its most abundant at low elevations in the study region (see Chapter 1), shows a greater trophic niche at the lowest sites. The species that are present in the lowest site (Prendes) have broader latitudinal distributions, reaching the North of Africa in the case of *B. terrestris* (Rasmont and Iserbyt 2010).

Previous studies have found a similar trend between differences in tongue lengths and overlap in resource use in bumblebee assemblages (Hanski 1982, Ranta et al. 1984, Goulson and Darvill 2004, Goulson et al. 2008*a*). Our results, obtained through a completely different approach in which spatial and temporal variation in resource use is integrated, support this trend and suggest a similar resource use among species of similar tongue lengths. Even though species with similar tongue lengths and high niche overlap co-occurred at high abundance in the study sites, these species should frequently interact as competitors when resources are scarce. The high overlap found at the lowest site (Prendes), where the species richness is low compared to medium elevations, might be explained by the presence of species of similar tongue length.

In conclusion, this study shows that bumblebees might have an optimum trophic niche width along the elevational gradient, which is closely related with its distributional range. It has been proposed that bumblebee decline is related to narrow climatic niches and that species may be most susceptible to decline in places where they are closest to their climatic range edges (Williams et al. 2007, 2009). This study also shows that bumblebee species have also narrower niches in their limit of their elevational ranges. Persistence of bumblebee populations might depend both on their relative position in their distribution range and the availability of floral resources as well as the interference with potential competitors (Williams et al. 2007, this study). In the face of the ongoing climate change, we could expect uphill and northward migrations of species (see Chapter 1), which may substantially affect the availability of floral resources and thus alter the trophic niches of mountain species (Layman et al. 2007*b*, Griffen et al. 2008, Jackson et al. 2012).

General Discussion

GENERAL DISCUSSION

Over recent decades, observations of range retractions in parallel with climate change and changing patterns in land use have been widely reported (e.g., Gaston et al. 2003, Thomas et al. 2006, Kosior et al. 2007, Chen at al. 2011*b*). The ideal approach to detect the biological impact of environmental changes is the resurveying of historical sites/transects (e.g., Moritz et al. 2008, Chen et al. 2009, Cameron et al. 2011). These resurveys have proved to be a particularly rich source of data in northern Europe, where there is a long tradition of observational records for many birds, butterfly and plants (e.g., Williams 1982, Parmesan et al. 1999, Thomas et al. 1999, Lenoir et al. 2008). However, studies from mountainous regions at lower latitudes are usually scarce (but see Stefanescu et al. 2004, Wilson et al. 2005).

In addition, most studies on the impacts of climate change on species' distribution shifts have been conducted at local scales and have only examined a small portion of the range, usually a single boundary (Parmesan 2007, Jump et al. 2009). There are, however, a few exceptions conducted at a scale that encompasses the entire range of the species, but this kind of observations are not common (e.g., Parmesan 1996, Parmesan et al. 1999). This is particularly important as different process govern each distribution margin: while biotic interactions determine species limits at warm margins, physical constraints might predominate at cool margins (Davis et al. 1998, Araújo and Luoto 2007). In this sense, elevational gradients provide a unique opportunity to study the entire elevational range of a species, as well as their lower and upper boundaries.

Warming-related range shifts and the community changes that may arise from such shifts are the primary concerns of this thesis, with a particular focus on the bumblebees in a mountain range in a temperate zone in North West of Spain. The existence of historical survey data for the area presents valuable opportunities

to examine how the changing environment may influence bumblebee communities (Obeso 1992, Fig. D.1).

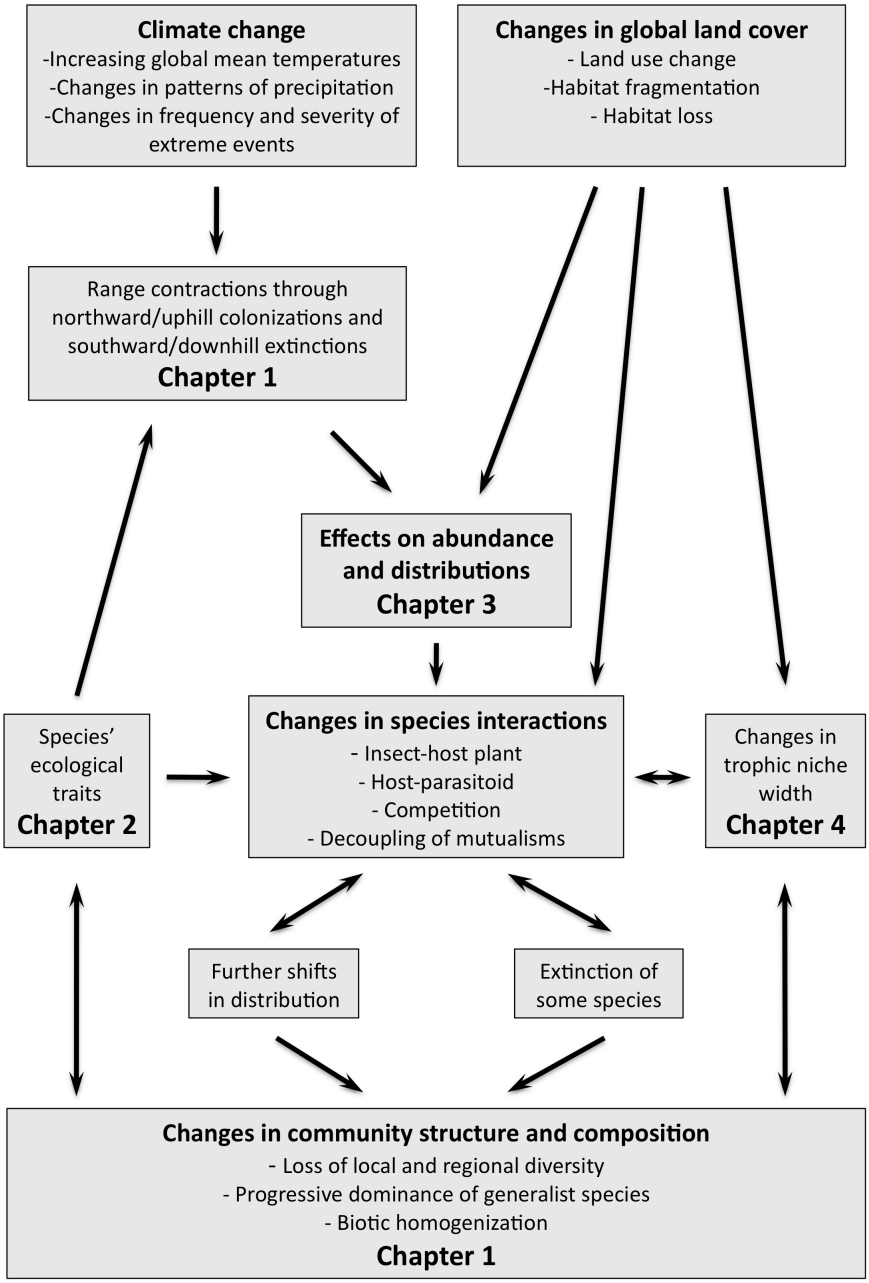

Figure D.1. Potential effect of climate change on individual bumblebee species and ways through which individual species responses could lead to changes in biodiversity and community composition. Individual species might potentially respond in four ways,resulting in changes in species interactions. These changes might then lead either to extinctions or to further shifts in ranges, ultimately leading to changes in the structure and composition of communities (modified from Hughes 2000 and Menéndez 2007).

Distributional shifts

This thesis provides evidence that bumblebee species have shifted uphill their upper or lower boundaries, resulting in narrower elevational ranges, over a period of 20 years (Chapter 1, Fig. D.1). Consistent with previous research on distributional shifts under environmental change, movement responses were species-specific, and differed in their magnitude and direction (Hickling et al. 2006, Lenoir et al. 2008, Chen et al. 2009). It has been suggested that species life history traits, such as a greater dispersal ability and ecological generalization, might modulate each species' realized response (Schweiger et al. 2008, Poÿry et al. 2009, Buckley et al. 2010, Angert et al. 2011). In this thesis, climatic niche breadth, though not other species' traits, mainly determined the individualistic response of bumblebee species (Chapter 2, Fig. D.1). Specifically, bumblebee species with broader climatic niches showed stronger changes in the climatic conditions at which they occurred in the two periods studied, as well as greater reductions in their elevational extent.

Species with broad climatic tolerance (*B. pratorum*, *B. mesomelas*, *B. wurflenii* and *B. lucorum*) shifted towards higher elevations, indicating a potential for colonizing newly suitable habitats (Chapters 1 and 2). However, these species also showed greater range contractions from one period to another (Chapters 1 and 2). Range contractions may be the result of species expanding the upper boundary of their range, and retracting the lower, at different rates (Chen et al. 2011*a*). For instance, lower boundaries might shift faster than upper boundaries if abiotic factors are hampering species from expanding uphill (e.g., because there is no more land available). Some mountain species (e.g., *B. wurflenii* and *B. mesomelas*) were however not able to expand their ranges uphill because they had already reached the summit of the mountainside suitable for them to thrive. As a consequence of this, these species did indeed show substantial range reductions in this study because they had also retreated their lower boundaries (Chapter 1).

A small group of species shifted their mean elevations and lower boundaries downhill. This group includes both medium and long-tongued species

(*B. hortorum*, *B. humilis* and *B. pascuorum*). Long-tongued bumblebees, like *B. hortorum* and *B. humilis*, show marked preferences for long corolla flowers (Pyke 1982, Goulson et al. 2005). It has been suggested that rare and declining species tend to be long tongued because they have narrower floral preferences (Goulson et al. 2005). Long tongued species were once common and abundant species in unimproved flower-rich grasslands rich in Fabaceae; an important and widespread habitat in Europe before the intensification of agricultural production. Most of these species have experienced marked decreases in their abundance and distribution in recent decades, which is associated with habitat loss and changes in farming practices (Goulson et al. 2005, Bommarco et al. 2011, Dupont et al. 2011). Although differences in tongue length did not explain the species-specific response observed in this thesis, stable isotope analysis showed that bumblebee species with similar tongue lengths show a higher degree of resource overlap, which suggests a similar resource use among these species (Chapters 2 and 4). In Chapter 1 and 3, we discuss the fact that potential changes in landscape structure are unlikely to have caused the distributional shifts in bumblebee species observed in the Cantabrian Range. However, availability of particular floral resources in lowlands may be the most important ecological factor for these species, and thus hindering them to shift uphill.

Community level changes

Colonizations and extinctions of species can have important consequences in community structure and composition (Memmott et al. 2007, Sheldon et al. 2011; Fig D.1). In particular, both shifts in species' range (Chapter 1) and individualistic responses among bumblebee species (Chapter 2) entailed substantial changes in community composition, characterized by a biotic homogenization of communities populating medium and upper elevations (Chapter 1). These changes in species composition and abundance of bumblebee species resulted in a marked shift in the composition of functional groups of bumblebees. As a consequence of the

generalized uphill shift of most bumblebee species, assemblages at lower elevations (0-900 m) became dominated by medium-tongued species, while both long and short-tongued species showed lower abundances (Chapter 1). Consequently, assemblages located at medium (900 – 1500 m) and upper elevations (> 1500 m) have become progressively dominated by short-tongued species (Chapter 1), which are often climatic generalists (Chapter 2). The historical trends in species composition of bumblebees described in this thesis, at medium and upper elevations, are similar to that of recent studies in Northern Europe (Bommarco et al. 2011, Dupont et al. 2011).

An important consequence of the arrival of colonizing species might be increased competition for food resources (Griffen et al. 2008). We know for instance that substantial niche reductions might occur after the arrival of invasive species (Griffen et al. 2008, Jackson et al. 2012) and reductions in resource availability (Layman et al. 2007*b*). Stable isotope analysis showed that the trophic niche of bumblebee species was smaller at medium elevations where species richness is considerably higher, than at lower or upper elevations (Chapter 4). Although species richness along the elevational gradient was similar from one period to another (Chapter 1), alterations in trophic niche width might still occur in the future if colonization and extinctions events alter interspecific interactions between coexisting species (Harrington et al. 1999, Schweiger et al. 2008).

Why bumblebees could be useful indicators of global change in mountain systems?

Most bumblebee species should be regarded as widely generalist species loosely associated with any particular habitat and visiting a large variety of floral resources (Westphal et al. 2003, Goulson and Darvill 2004, Goulson et al. 2006). For instance, some species like *B. terrestris* can be remarkably common even in gardens and suburban areas, and others bumblebee species occupy markedly different biotopes in different parts of their range (Goulson et al. 2006, Goulson

2010). Common species (e.g. *B. terrestris*, *B. pratorum*) can be also very opportunistic in the choice of their nest site (Kells and Goulson 2003). Consistent with this, the distribution and abundance of bumblebee species in the Cantabrian Range was mainly related to climatic variables, and no significant relationship was found between any bumblebee species and particular habitat types (Chapter 3).

Despite their generalist behaviour, some bumblebee species have drastically regressed in large regions of Western Europe and Northern America (Williams 1982, Kosior et al. 2007, Cameron et al. 2011). There are two predominant hypothesis which may explain the differences in susceptibility in bumblebee species: (1) diet specialization and decline of preferred food plants (Goulson et al. 2005, 2008*b*, Kleijn and Raemakers 2008), and (2) a greater climatic specialization and proximity to the edges of their climatic ranges (Williams et al. 2007, 2009). Consequently, it seems clear that specialization is a key factor determining decline and vulnerability of bumblebees, as it have been revealed in studies with animals and plants (Clavel et al. 2012).

It is widely accepted that the decline in abundance and distribution of bumblebee species has been related to changes in land use due to agricultural intensification, and subsequent decline of preferred food resources (Kosior et al. 2007, Goulson et al. 2008*b*, Kleijn and Raemakers 2008). Changing climate conditions, on the other hand, might have a disproportionate effect in mountainous regions where agricultural practices are generally less intensive (e.g., Goulson 2008*a*). Our results suggest that the trophic niche of bumblebee species is context-dependent and is determined by interference with potential competitors (Chapter 4). Both short and long tongued species showed variation in their trophic niche width related with species richness in the assemblage considered. More specifically, species showed narrower trophic niches in species-rich assemblages. Thus, susceptibility to environmental changes is not determined by trophic specialization, at least in our study system. However, climatic tolerance strongly determined each individual species' response (Chapter 2).

At a continental scale, bumblebees' European climatic niches were determined by temperature and, in a lesser extent, by moisture gradients (Chapter 2). This is in line with previous studies on climatic niche and abundance and distribution of bumblebee species (Banaszak 1996, Hatfield and LeBuhn 2007, Williams et al. 2007). Bumblebees are cool-adapted pollinators, feeding almost exclusively on pollen and nectar, and thus expected to depend on both temperature conditions and water availability (Heinrich 1979). In this thesis, all bumblebee species (except for *B. hortorum*), tracked localities *ca.* 1.3°C cooler after two decades, whereas rainfall conditions had lower importance in determining bumblebees' response (Chapter 2). There may be at least three possible explanations for this pattern of response. First, the relative change on rainfall patterns in the study region did not exhibit clear tendencies during the last three decades (IPCC 2007, Álvarez et al. 2009). Second, the lack of a clear precipitation gradient along elevation in the study region is relatively common in mountainous regions with rugged topography, and thus studies carried out on these systems can neither prove nor disapprove any effect of precipitation patterns on species range shifts (Körner 2007). Instead, a great diversity of geographic responses should not only be predicted, but also expected, especially in topographically complex environments (Peterson et al. 2002). Third, temperature variables were much more important in defining the climatic niche of bumblebee' species and therefore is a more plausible driver of bumblebee distributional shifts.

In conclusion, this thesis provides evidence of the suitability of bumblebee species as biological indicators of global environmental change. Bumblebees in the Cantabrian Range have shifted uphill at both species and community level in line with fingerprints expected under climate change and consistent with a regional warming of *ca*. 0.8°C (Chapter 1). Furthermore, climate sensibility and climatic variables determined bumblebee response and distribution, respectively (Chapters 2 and 3). Finally, bumblebee assemblages in mountain systems might be especially useful to study changes in community structure and composition we may expect under environmental changes (Chapter 4).

Implications for species persistence and conservation

Mountain areas are among the regions most affected by climate change, and have been increasingly affected by loss of biodiversity due to expansion and intensification of land use (Korner and Oshawa 2004). With even increased climate warming predicted for mountain systems, habitats for organisms adapted to cold environments will be curtailed (Nogués-Bravo et al. 2007). Moreover, species already inhabiting summit regions will be in a very difficult situation because habitable area might be particularly reduced (Wilson et al. 2005, Chen et al. 2011*a*). Given that ongoing and expected climate changes are much faster than what evolution and migration are commonly able to cope with (Parmesan 2006; but see Hof et al. 2011), further disruptions in species interactions might be expected (e.g., Sheldon et al. 2011, Fig. D.1.). Therefore, more severe range contractions are expected on range-restricted species such as mountain top species in the directions predicted from global warming (Parmesan 2006, Fig. D.1.).

In this sense, protecting areas across environmental gradients has been suggested to be an effective conservation approach because these areas would act as climate refugia (Bush 2002, Killeen and Solórzano 2008). On the other hand, the study and characterization of the species' ecological requirements should be essential to understand and predict both species responses to environmental change and changes in community structure and composition (Bolnick et al. 2011). More specifically, the study of the ecological niche should be an important approach in the attempt to identify differences in the response rates of different species, as well as to identify vulnerable subgroups and to prioritize conservation strategies (Koh 2004, Mattila et al. 2008, Jiguet 2010). Finally, it is worth noting that rear edge populations (such as many bumblebee species in the Cantabrian Range) are of critical importance to both understand species' response to environmental changes and species persistence because of their ecological and genetic differentiation (Hampe and Petit 2005, Blanco-Fontao et al. 2010).

Conclusions

1. Bumblebee species showed and overall trend to shift uphill their upper- or lower-elevation boundaries, resulting in narrower elevational ranges over a period of 20 years. The changes in elevational ranges were mainly related to increases in the lower limit of species distribution, rather to decreases in their upper elevational limit.

2. Species turnover was associated with colonization and extinction events and also with variability in the abundance of the species. Extinction events were especially relevant at medium elevations (900 – 1500 m), while only upper communities (> 1500 m) had a positive net outcome between colonization and extinction events.

3. The combination of colonizations and extinctions events along the elevational gradient resulted in the homogenization of bumblebee assemblages, especially between medium and upper elevations.

4. Climatic tolerance mainly determined each individual species' response. Bumblebee species with broad climatic niches showed stronger changes in the climatic conditions at which they occurred from one period to another. Additionally, bumblebee species with broad climatic niches also showed greater decreases in their elevational extent from one period to another. Accordingly, climatic niche breadth might be a particularly informative ecological trait for understanding and predicting the response of different species to climate change and, in turn, their distribution range shifts.

5. Bumblebee presence and abundance patterns in the Cantabrian Range were mainly determined by climatic variables (notably temperature), rather that land-cover variables.

6. Six bumblebee species showed climate tracking rather that responses to land cover changes. However, the abundance of three bumblebee species

was determined by land-cover variables (*B. sichelii*, *B. soroeensis*, *B. wurflenii*).

7. Bumblebee species showed narrower trophic niches and a greater degree of niche overlap between species pairs in species-rich communities, suggesting a more specialized diet and stronger competition in those communities.

8. There was a negative relationship between niche overlap and the difference in tongue length between species pairs, which suggest a similar resource use among species of similar tongue length.

Conclusiones

CONCLUSIONES

1. Tras un periodo de 20 años, las especies de abejorros de la Cordillera Cantábrica mostraron un desplazamiento hacia zonas más altas en sus límites de distribución altitudinal. En consecuencia, el rango altitudinal de la mayoría de las especies estudiadas disminuyó considerablemente. Esta disminución en el rango altitudinal ocupado fue debida a incrementos en el límite inferior de distribución de las especies, en lugar de cambios en el límite superior.

2. El remplazo de especies a lo largo del gradiente altitudinal se produjo como consecuencia de eventos de colonización y extinción de las distintas especies. Las extinciones fueron predominantes a altitudes medias (900 – 1500 m), mientras que únicamente las comunidades de zonas altas (> 1500 m) presentaron un balance neto positivo entre colonizaciones y extinciones.

3. En conjunto, las colonizaciones y extinciones a lo largo del gradiente altitudinal dieron como resultado la homogenización de las comunidades de abejorros entre zonas medias y altas.

4. La respuesta de las distintas especies estuvo determinada por su tolerancia climática. Los abejorros con mayor amplitud de nicho climático mostraron cambios más pronunciados en las condiciones climáticas a las que se encontraban entre ambos periodos. Asimismo, las especies con mayor amplitud de nicho climático mostraron una mayor disminución en el rango altitudinal ocupado. Por tanto, la amplitud de nicho climático constituye un rasgo ecológico particularmente informativo para comprender y predecir la respuesta de las especies de abejorros al cambio climático y, en consecuencia, los potenciales cambios en los rangos de distribución geográfica.

5. La presencia y abundancia de las especies de abejorros en la Cordillera Cantábrica estuvo determinado predominantemente por variables climáticas (en concreto temperatura) en lugar de variables de paisaje.

6. Seis especies de abejorros mostraron una respuesta a cambios en las condiciones climáticas en lugar de cambios en las variables de paisaje. Por otra parte, los cambios en abundancia de tres especies de abejorros respondieron a cambios en variables de paisaje (*B. sichelii*, *B. soroensis*, *B. wurflenii*).

7. Las distintas especies de abejorros presentan una menor amplitud de nichos trófico en localidades caracterizadas por una elevada riqueza de especies. Además, el solapamiento de nicho fue más elevado en dichas localidades. Estos resultados sugieren dietas más especializadas y una mayor competencia interspecífica en esas localidades, así como mayor similitud en el uso de recursos.

8. Especies similares en cuanto a la longitud de la probóscides presentan mayor solapamiento de nicho trófico, lo que sugiere un uso similar de recursos tróficos entre estas especies.

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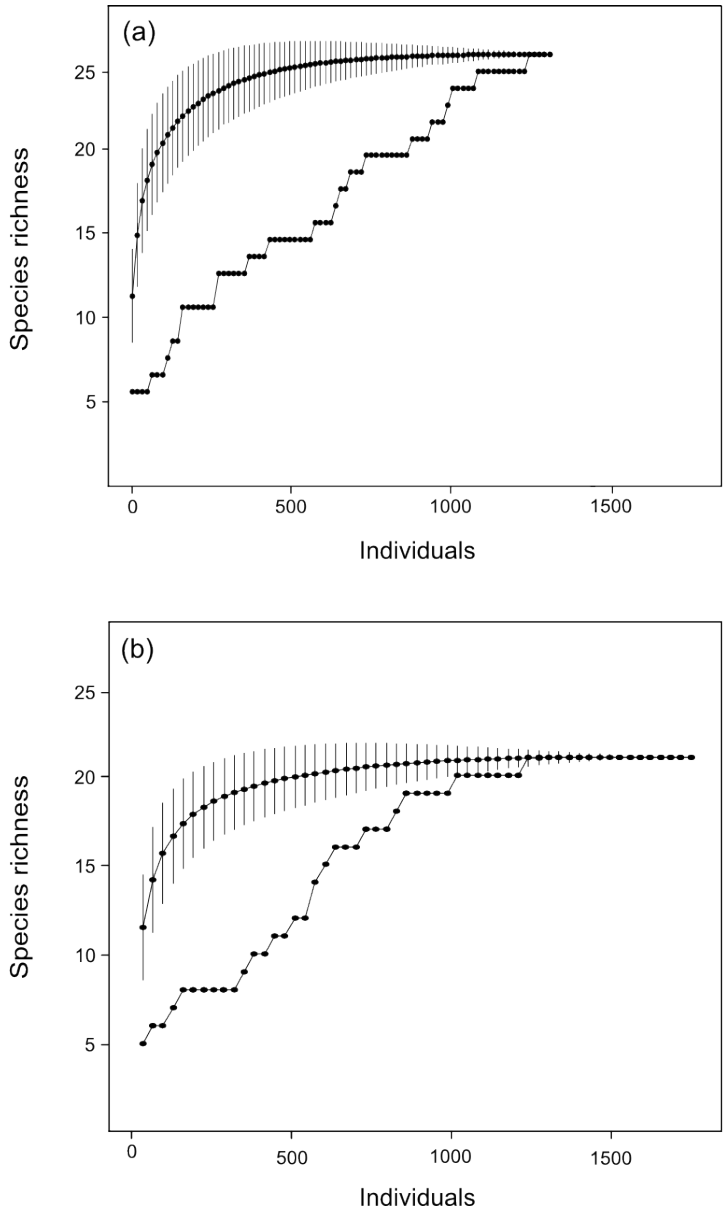
Appendix

APPENDIX

APPENDIX 1.1. Rarefaction (smooth curves) and accumulation (jagged curves) curves of species richness based on individuals in the 1988-1989 (a) and 2007-2009 (b) periods. Data was pooled following the elevational gradient.



APPENDIX 1.2. Supporting results of one-sample *t*-tests for shifts of average elevations, lower and upper boundaries and elevational extent of bumblebee species (*Bombus* spp.) in the Cantabrian Range.

Species	*n*	d.f.	Mean altitude			Lower boundary			Upper boundary			Elevational extents		
			t	95% confidence interval		*t*	95% confidence interval		*t*	95% confidence interval		*t*	95% confidence interval	
				Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper
B. cullumanus ‡	---	---	---	---	---	---	---	---	---	---	---	---	---	---
B. gerstaeckeri ‡	---	---	---	---	---	---	---	---	---	---	---	---	---	---
B. hortorum	30	499	-86.6	-534.5	-510.8	-5.6	-10.8	-5.2	-1.0	-21.1	6.8	-1.9	-33.9	0.1
B. humilis	10	499	-79.3	-265.6	255.6	-28.8	-118.1	-102.9	42.8	548.2	600.8	59.5	697.4	745.1
B. hypnorum ‡	---	---	---	---	---	---	---	---	---	---	---	---	---	---
B. inexpectatus ‡	---	---	---	---	---	---	---	---	---	---	---	---	---	---
B. jonellus	25	499	20.2	79.2	96.9	-73.8	-631.7	-599.1	229.2	202.2	205.7	108	806.4	836.3
B. laesus ‡	---	---	---	---	---	---	---	---	---	---	---	---	---	---
B. lapidarius	30	499	64.7	282.7	300.4	30.2	533.2	607.2	5.0	37.9	85.6	-25.1	-570.4	-487
B. lucorum	30	499	73.5	384.7	405.8	14.4	217.9	286.4	28.6	254.6	292.2	-0.1	-44.4	38.4
B. mesomelas	30	499	15.9	58.8	75.3	81.1	627.3	658.5	-137.0	-2.1	0.3	-81.1	-641.9	-611.6
B. monticola	10	499	-86.2	-235.3	224.8	63.1	173.1	184.2	-8.1	-40.3	-24.6	-43.9	-216.1	-197.6
B. mucidus ‡	---	---	---	---	---	---	---	---	---	---	---	---	---	---
B. muscorum	15	499	132.1	707.1	728.5	25.5	56.8	66.3	38.8	245.1	271.2	28.5	187.2	214.8
B. pascuorum	15	499	-46.4	-205.8	-189.1	-15.8	-19.1	-14.8	17.3	186.9	234.6	18.7	215.4	265.81
B. pratorum	15	499	55.4	382.5	410.6	14.1	36.5	48.4	48.5	207.9	225.4	27.3	154.5	178.4
B. ruderarius	30	499	32.3	101.4	114.6	17.3	381.3	478.6	2.9	7.1	38.7	-15.1	-438.1	-337.1
B. ruderatus ‡	---	---	---	---	---	---	---	---	---	---	---	---	---	---
B. sichelii	30	499	-30.3	-154.7	-135.9	-5.8	-46.0	-22.7	-13.8	-85.9	-64.6	-4.9	-55.7	-24.6
B. soroeensis	30	499	-17.9	-110.8	-96.0	30.5	212.1	241.5	-11.1	136.7	-95.6	-24.1	-347.9	-295.5
B. subterraneus ‡	---	---	---	---	---	---	---	---	---	---	---	---	---	---
B. sylvarum	30	499	18.6	98.3	121.5	21.1	3.1	3.7	-4.2	-75.6	-27.7	-1.5	-50.6	5.3
B. terrestris	30	499	-8.6	-83.2	-52.5	15.3	7.1	9.2	0.0	-27.2	26.9	0.1	-24.8	28.1
B. wurflenii	30	499	72.3	295.9	312.4	89.4	1072.2	1120.5	-17.9	-200.3	-160.7	-77.4	-1291	-1226.9

n, number of individuals subsampled for each species and included in the analysis.
‡ *t*-tests were not performed because number of individuals was below ten in at least one period.

APPENDIX 2.1. General linear models (one sample *t*-test, H_0: μ = 0) predicting rate of thermal (RTC) and rainfall (RPC) change of each individual species between 1988-1989 and 2007-2009 in the Cantabrian Range. Analysis were made after a bootstrapping procedure (resample was repeated 500 times).

			Rate of thermal change (%)				Rate of precipitation change (%)			
	n	**d.f.**	***t***	**95% confidence interval**		**mean (SE)**	***t***	**95% confidence interval**		**mean (SE)**
Species				**Lower**	**Upper**			**Lower**	**Upper**	
B. hortorum	40	499	211.17	5.72	56.01	5.98 (2.8*	4.84	0.48	1.1	0.8 (2.6)
B. jonellus	20	499	7.55	2.95	3.15	3.09 (2.2)*	-6.76	0.93	1.9	1.31 (3.07)*
B. lapidarius	60	499	11.87	28.75	29.22	28.92 (2.3)***	25.96	2.33	2.7	2.52 (1.5)*
B. lucorum	60	499	-148.95	26.23	26.68	26.44 (3.4)***	68.47	9.02	9.6	9.28 (2.1)***
B. mesomelas	50	499	80.23	29.92	30.55	30.33 (2.8)***	-55.86	6.77	7.3	7.02 (3.9)*
B. muscorum	15	499	189.35	16.61	16.99	16.87 (2.4)**	15.50	3.22	4.2	3.69 (3.7)**
B. pratorum	15	499	100.84	25.15	25.52	25.36 (2.6)***	11.84	2.16	3	2.59 (3.4)*
B. ruderarius	20	499	34.46	11.78	12.01	11.95 (2.1)*	14.01	3.27	4.3	3.81 (4.3)**
B. sichelii	30	499	-30.55	3.55	3.65	3.74 (2.1)	-3.76	0.59	1.9	1.25 (0.0)
B. soroeensis	60	499	41.17	1.77	1.95	1.87 (2.3)*	-21.89	2.95	3.5	3.25 (2.3)**
B. terrestris	150	499	101.12	23.13	23.62	23.34 (2.5)***	-92.39	4.58	4.8	4.68 (2.1)*
B. wurflenii	30	499	-146.51	30.31	30.51	30.61 (2.4)***	-62.82	9.97	11	10.30 (2.3)**

n = the number of subsampled specimens of each individual species.
Significance levels are based on one-sample *t*-tests ([H_0: μ = 0 (no change)]): *P < 0.05; **P < 0.01; ***P < 0.001.

APPENDIX 2.2. Pearson correlation coefficients for correlation among predictor variables.

	Thermal niche breadth (PC1)	**Rainfall niche breadth (PC2)**	**Tongue length**	**T_{ex}**	**Wingloading**
Rainfall niche breadth (PC2)	0.65				
Tongue length	0.07	-0.16			
T_{ex}	0.74	0.71	-0.17		
Wingloading	-0.04	-0.06	-0.17	-0.38	
Wing aspect	0.64	0.30	-0.33	0.31	0.35

T_{ex} = Temperature excess (difference between thoracic temperature and ambient temperature).

APPENDIX 3.1. Multiple correlations among topoclimatic and land-cover variables extracted at 100 m spatial scale. Tables show two-way Spearman rank correlations (lower left) and associated probabilities (upper right). High association (|r|>0.70) between pairs of predictor variables was used to identify correlated groups of variables that had to be reduced to a single one, are highlighted in bold-face. Habitat size = mean size of all suitable habitat patches for bumblebees (grassland + shrubland land-covers); Habitat isolation = edge-to-edge mean distance between all habitat patches in a radius of 3000 m surrounding each locality; *H* = Shannon-Wiener diversity index of land-cover.

	Altitude	**Forest**	**Shrub**	**Rock**	**Grassland**	**Slope100**	**Slope3000**	***H***	**Temp**	**Rainfall**	**Size**	**Isolation**
Altitude		0.0033	0.0107	0.0665	0.2384	0.0853	0.7778	0.1118	0.0000	0.5434	0.5437	0.1456
Forest	-0.4532		0.0002	0.3914	0.0771	0.6617	0.4062	0.6793	0.0043	0.7650	0.6789	0.3452
Shrub	0.3995	-0.5638		0.1386	0.0174	0.3124	0.6238	0.3523	0.0118	0.5342	0.4275	0.5846
Rock	0.2930	-0.1393	-0.2384		0.4592	0.0112	0.4719	0.7916	0.0416	0.7054	0.7945	0.7934
Grassland	-0.1907	-0.2827	-0.3740	-0.1204		0.0300	0.0947	0.2184	0.1413	0.6803	0.0412	0.0450
Slope100	0.2755	-0.0714	0.1638	**0.7671**	-0.3434		0.5681	0.1567	0.0842	0.9159	0.5472	0.4267
Slope3000	-0.0461	0.1350	-0.0800	0.1171	-0.2679	0.0930		0.4963	0.9313	0.2742	0.4356	0.0326
H	0.2553	0.0674	0.1510	0.0431	-0.1990	0.2282	-0.1108		0.1189	0.5262	0.4278	0.3176
Temp	**-0.9923**	0.4417	-0.3945	-0.3237	0.2368	-0.2765	-0.0141	-0.2506		0.5290	0.0567	0.4804
Rainfall	-0.0990	0.0488	0.1013	0.0617	-0.0672	0.0172	0.1771	0.1032	0.1025		0.2313	0.2168
Size	0.0678	0.2186	0.3450	0.0087	**0.7204**	0.0000	0.2341	-0.1865	0.2315	0.2134		0.2852
Isolation	0.0876	0.0645	0.0987	0.1340	**0.7187**	0.1254	0.1643	-0.2132	0.1654	0.0887	0.1231	

APPENDIX 3.2. AUC scores (± SE) used to predict the presence of bumblebees (*Bombus* spp.) for each land-cover variables at increasingly 100, 250, 500, 750, 1500, 2000 and 3000 m nested neighbourhood distances (see Fig. 3.1 in the main text). We highlighted in bold face the higher value of AUC scores (± SE) for each variable at each spatial scale, which were later included in the GLMs testing the effects of topoclimatic and land-cover variables on species presence (see Table 3.1 in the main text). *H* = Shannon-Wiener diversity index of land-cover.

	Spatial scale						
Species	**100m**	**250m**	**500m**	**750m**	**1500m**	**2000m**	**3000m**
B. hortorum							
Forest	0.65 ± 0.09	**0.73 ± 0.08**	0.65 ± 0.08	0.62 ± 0.09	0.66 ± 0.09	0.66 ± 0.10	0.65 ± 0.10
Shrubland	**0.81 ± 0.07**	0.74 ± 0.08	0.65 ± 0.08	0.73 ± 0.08	0.75 ± 0.07	0.76 ± 0.07	0.70 ± 0.08
Grassland	0.64 ± 0.08	**0.74 ± 0.08**	0.61 ± 0.09	0.73 ± 0.09	0.50 ± 0.11	0.51 ± 0.09	0.70 ± 0.09
Diversity (*H*)	0.65 ± 0.09	**0.73 ± 0.08**	0.66 ± 0.09	0.71 ± 0.09	0.50 ± 0.10	0.51 ± 0.11	0.64 ± 0.11
B. humilis							
Forest	0.68 ± 0.08	0.71 ± 0.08	0.71 ± 0.07	**0.76 ± 0.08**	0.74 ± 0.09	0.75 ± 0.09	0.68 ± 0.08
Shrubland	**0.69 ± 0.07**	0.61 ± 0.08	0.57 ± 0.09	0.62 ± 0.09	0.68 ± 0.09	0.68 ± 0.09	0.69 ± 0.08
Grassland	**0.69 ± 0.07**	0.66 ± 0.09	0.64 ± 0.10	0.59 ± 0.10	0.68 ± 0.09	0.68 ± 0.09	0.69 ± 0.08
Diversity (*H*)	**0.67 ± 0.09**	0.66 ± 0.09	0.64 ± 0.10	0.59 ± 0.10	0.60 ± 0.09	0.63 ± 0.09	0.59 ± 0.10
B. jonellus							
Forest	0.62 ± 0.09	0.57 ± 0.08	0.61 ± 0.08	0.54 ± 0.10	0.59 ± 0.09	0.57 ± 0.11	**0.67 ± 0.08**
Shrub	0.62 ± 0.09	0.50 ± 0.10	0.61 ± 0.08	0.64 ± 0.10	0.65 ± 0.11	0.64 ± 0.10	**0.67 ± 0.08**
Grassland	0.56 ± 0.08	0.50 ± 0.10	0.65 ± 0.11	0.64 ±0.10	0.65 ± 0.11	0.64 ± 0.10	**0.65 ± 0.10**
Diversity (*H*)	0.62 ± 0.10	0.54 ± 0.10	0.65 ± 0.11	0.54 ± 0.10	0.59 ± 0.10	0.59 ± 0.11	**0.65 ± 0.10**
B. lapidarius							
Forest	0.56 ± 0.09	0.59 ± 0.09	0.53 ± 0.09	0.57 ± 0.09	0.60 ± 0.09	0.63 ± 0.09	**0.64 ± 0.08**
Shrubland	0.56 ± 0.09	0.59 ± 0.09	0.53 ± 0.09	0.56 ± 0.09	0.60 ± 0.09	0.63 ± 0.09	**0.64 ± 0.08**
Grassland	0.56 ± 0.08	0.54 ± 0.08	0.51 ± 0.09	0.55 ± 0.09	0.53 ± 0.09	0.51 ± 0.09	**0.60 ± 0.09**
Diversity (*H*)	0.56 ± 0.08	0.54 ± 0.08	0.53 ± 0.09	0.57 ± 0.09	0.59 ± 0.09	0.56 ± 0.09	**0.60 ± 0.09**
B. lucorum							
Forest	0.64 ± 0.08	0.63 ± 0.08	0.56 ± 0.09	0.64 ± 0.09	0.62 ± 0.09	**0.65 ± 0.09**	0.64 ± 0.09
Shrubland	**0.67 ± 0.08**	0.63 ± 0.09	0.56 ± 0.09	0.65 ± 0.09	0.64 ± 0.09	0.62 ± 0.09	0.62 ± 0.09
Grassland	**0.67 ± 0.08**	0.56 ± 0.10	0.56 ± 0.09	0.50 ± 0.10	0.50 ± 0.10	0.50 ± 0.10	0.62 ± 0.09
Diversity (*H*)	**0.66 ± 0.09**	0.56 ± 0.10	0.50 ± 0.10	0.50 ± 0.10	0.50 ± 0.10	0.50 ± 0.10	0.50 ± 0.10
B. mesomelas							
Forest	0.72 ± 0.04	0.74 ± 0.04	0.51 ± 0.09	**0.79 ± 0.06**	0.79 ± 0.06	0.78 ± 0.07	0.71 ± 0.08
Shrubland	0.53 ± 0.09	0.51 ± 0.09	0.51 ± 0.09	**0.61 ± 0.08**	0.51 ± 0.09	0.55 ± 0.10	0.56 ± 0.10
Grassland	0.54 ± 0.10	0.51 ± 0.09	0.66 ± 0.08	**0.70 ± 0.08**	0.65 ± 0.09	0.65 ± 0.09	0.57 ± 0.10
Diversity (*H*)	0.54 ± 0.10	**0.57 ± 0.10**	0.52 ± 0.10	0.55 ± 0.10	0.52 ± 0.10	0.54 ± 0.10	0.51 ± 0.10

APPENDIX 3.2. Continued.

	Spatial scale						
Species	**100m**	**250m**	**500m**	**750m**	**1500m**	**2000m**	**3000m**
B. muscorum							
Forest	**0.68 ± 0.11**	0.56 ± 0.09	0.61 ± 0.10	0.62 ± 0.12	0.56 ± 0.12	0.53 ± 0.12	0.55 ± 0.11
Shrubland	**0.59 ± 0.10**	0.68 ± 0.11	0.56 ± 0.10	0.62 ± 0.12	0.56 ± 0.12	0.50 ± 0.13	0.57 ± 0.11
Grassland	0.56 ± 0.09	0.54 ± 0.11	0.57 ± 0.11	0.54 ± 0.09	**0.63 ± 0.09**	0.63 ± 0.09	0.57 ± 0.11
Diversity (*H*)	**0.68 ± 0.11**	0.54 ± 0.11	0.57 ± 0.11	0.54 ± 0.11	0.50 ± 0.12	0.53 ± 0.12	0.55 ± 0.11
B. pratorum							
Forest	0.53 ± 0.07	0.60 ± 0.09	0.73 ± 0.09	0.68 ± 0.06	**0.76 ± 0.06**	0.76 ± 0.07	0.70 ± 0.08
Shrubland	0.63 ± 0.09	0.51 ± 0.10	0.60 ± 0.10	0.53 ± 0.10	0.61 ± 0.11	0.65 ± 0.10	**0.70 ± 0.08**
Grassland	0.54 ± 0.09	0.59 ± 0.08	0.65 ± 0.07	0.67 ± 0.10	**0.68 ± 0.09**	0.66 ± 0.09	0.64 ± 0.09
Diversity (*H*)	0.54 ± 0.09	0.50 ± 0.10	0.55 ± 0.10	0.52 ± 0.11	0.59 ± 0.11	0.60 ± 0.11	**0.63 ± 0.10**
B. ruderarius							
Forest	0.53 ± 0.09	0.55 ± 0.07	0.54 ± 0.09	0.54 ± 0.09	0.51 ± 0.08	0.60 ± 0.09	**0.65 ± 0.09**
Shrubland	0.62 ± 0.08	0.60 ± 0.08	0.53 ± 0.09	0.62 ± 0.09	**0.63 ± 0.09**	0.55 ± 0.09	0.53 ± 0.09
Grassland	0.60 ± 0.06	0.51 ± 0.09	0.53 ± 0.09	0.57 ± 0.09	**0.63 ± 0.09**	0.51 ± 0.09	0.50 ± 0.09
Diversity (*H*)	0.53 ± 0.09	0.51 ± 0.09	0.56 ± 0.09	**0.57 ± 0.09**	0.51 ± 0.09	0.52 ± 0.09	0.53 ± 0.09
B. sichelii							
Forest	0.61 ± 0.07	0.63 ± 0.07	0.63 ± 0.08	0.59 ± 0.08	**0.64 ± 0.08**	0.61 ± 0.08	0.59 ± 0.09
Shrubland	0.56 ± 0.08	0.51 ± 0.09	**0.60 ± 0.09**	0.55 ± 0.09	0.53 ± 0.09	0.54 ± 0.09	0.52 ± 0.09
Grassland	0.51 ± 0.09	0.59 ± 0.08	0.54 ± 0.09	0.60 ± 0.08	0.63 ± 0.08	0.64 ± 0.08	**0.67 ± 0.08**
Diversity (*H*)	0.51 ± 0.09	0.50 ± 0.09	0.54 ± 0.09	**0.55 ± 0.09**	0.51 ± 0.09	0.50 ± 0.09	0.52 ± 0.09
B. soroeensis							
Forest	**0.66 ± 0.09**	0.51 ± 0.07	0.53 ± 0.08	0.52 ± 0.08	0.53 ± 0.08	0.55 ± 0.09	0.59 ± 0.09
Shrubland	**0.66 ± 0.09**	0.58 ± 0.08	0.57 ± 0.09	0.61 ± 0.09	0.60 ± 0.09	0.59 ± 0.09	0.59 ± 0.09
Grassland	0.60 ± 0.08	0.58 ± 0.08	0.57 ± 0.09	0.53 ± 0.10	0.57 ± 0.09	0.52 ± 0.09	**0.67 ± 0.08**
Diversity (*H*)	**0.60 ± 0.08**	0.52 ± 0.09	0.51 ± 0.09	0.53 ± 0.10	0.50 ± 0.09	0.52 ± 0.09	0.56 ± 0.09
B. terrestris							
Forest	0.68 ± 0.08	0.67 ± 0.08	**0.77 ± 0.07**	0.61 ± 0.09	0.65 ± 0.09	0.64 ± 0.09	0.51 ± 0.09
Shrubland	0.68 ± 0.08	0.67 ± 0.08	**0.77 ± 0.07**	0.76 ± 0.07	0.65 ± 0.09	0.64 ± 0.09	0.58 ± 0.09
Grassland	**0.65 ± 0.06**	0.56 ± 0.07	0.64 ± 0.07	0.65 ± 0.07	0.58 ± 0.09	0.59 ± 0.09	0.60 ± 0.09
Diversity (*H*)	0.61 ± 0.08	**0.67 ± 0.08**	0.51 ± 0.09	0.61 ± 0.09	0.61 ± 0.09	0.55 ± 0.09	0.51 ± 0.09
B. wurflenii							
Forest	0.63 ± 0.06	0.65 ± 0.06	0.54 ± 0.10	0.59 ± 0.09	0.66 ± 0.08	0.72 ± 0.08	**0.77 ± 0.08**
Shrubland	0.59 ± 0.09	0.59 ± 0.10	0.60 ± 0.11	0.60 ± 0.09	0.54 ± 0.10	0.58 ± 0.10	**0.62 ± 0.10**
Grassland	0.59 ± 0.09	0.59 ± 0.10	0.60 ± 0.11	0.59 ± 0.09	**0.60 ± 0.11**	0.57 ± 0.11	0.55 ± 0.10
Diversity (*H*)	0.54 ± 0.10	**0.64 ± 0.09**	0.50 ± 0.09	0.51 ± 0.10	0.55 ± 0.10	0.55 ± 0.10	0.55 ± 0.10

APPENDIX 3.3. Spearman (r_s) correlation scores (± SE) used to predict bumblebee (*Bombus* spp.) species' abundance for each land-cover variables at increasingly 100, 250, 500, 750, 1500, 2000 and 3000 m nested spatial scales (see Fig. 3.1 in the main text). Variables in bold face were the scale-dependent land-cover features included in latter analyses (see Table 3.2 in the main text). For further conventions, see Appendix 3.3.

	Spatial scale						
Species	**100m**	**250m**	**500m**	**750m**	**1500m**	**2000m**	**3000m**
B. hortorum							
Forest	**0.34 ± 0.14**	0.17 ± 0.15	0.16 ± 0.15	0.13 ± 0.15	0.05 ± 0.15	0.04 ± 0.15	0.02 ± 0.15
Shrubland	0.32 ± 0.14	**0.46 ± 0.12**	0.35 ± 0.14	0.43 ± 0.13	0.42 ± 0.14	0.40 ± 0.13	0.31 ± 0.14
Grassland	**0.50 ± 0.15**	0.44 ± 0.15	0.29 ± 0.15	0.41 ± 0.15	0.43 ± 0.15	0.44 ± 0.15	0.34 ± 0.15
Diversity (*H*)	0.29 ± 0.14	0.36 ± 0.13	0.27 ± 0.14	0.36 ± 0.13	**0.37 ± 0.13**	0.35 ± 0.14	0.30 ± 0.14
B. humilis							
Forest	0.22 ± 0.15	0.33 ± 0.15	0.03 ± 0.15	0.20 ± 0.15	0.21 ± 0.15	0.09 ± 0.15	**0.22 ± 0.15**
Shrubland	**0.33 ± 0.14**	0.15 ± 0.15	0.07 ± 0.15	0.13 ± 0.15	0.03 ± 0.15	0.04 ± 0.15	0.15 ± 0.15
Grassland	0.12 ± 0.15	0.16 ± 0.15	0.12 ± 0.15	0.02 ± 0.15	0.18 ± 0.15	**0.20 ± 0.15**	0.14 ± 0.15
Diversity (*H*)	0.15 ± 0.15	0.04 ± 0.15	0.20 ± 0.15	0.00 ± 0.15	0.07 ± 0.15	**0.20 ± 0.15**	0.12 ± 0.15
B. jonellus							
Forest	0.01 ± 0.15	0.05 ± 0.15	0.08 ± 0.15	0.15 ± 0.15	0.10 ± 0.15	0.08 ± 0.15	**0.10 ± 0.15**
Shrubland	0.00 ± 0.15	**0.14 ± 0.15**	0.04 ± 0.15	0.08 ± 0.15	0.04 ± 0.15	0.03 ± 0.15	0.05 ± 0.15
Grassland	0.14 ± 0.15	0.07 ± 0.15	**0.21 ± 0.15**	0.06 ± 0.15	0.06 ± 0.15	0.06 ± 0.15	0.01 ± 0.15
Diversity (*H*)	0.06 ± 0.15	**0.08 ± 0.15**	0.08 ± 0.15	0.03 ± 0.15	0.01 ± 0.15	0.03 ± 0.15	0.01 ± 0.15
B. lapidarius							
Forest	**0.27 ± 0.14**	0.23 ± 0.15	0.12 ± 0.15	0.20 ± 0.15	0.22 ± 0.15	0.08 ± 0.15	0.02 ± 0.15
Shrubland	0.19 ± 0.15	**0.36 ± 0.15**	0.21 ± 0.15	0.19 ± 0.15	0.14 ± 0.15	0.01 ± 0.15	0.23 ± 0.15
Grassland	0.21 ± 0.15	0.10 ± 0.15	0.01 ± 0.15	0.13 ± 0.15	0.11 ± 0.15	**0.23 ± 0.15**	0.04 ± 0.15
Diversity (*H*)	0.13 ± 0.15	0.16 ± 0.15	0.21 ± 0.15	0.05 ± 0.15	0.14 ± 0.15	0.20 ± 0.15	**0.30 ± 0.15**
B. lucorum							
Forest	**0.33 ± 0.14**	0.26 ± 0.14	0.05 ± 0.15	0.0 ± 0.15	0.04 ± 0.15	0.05 ± 0.15	0.08 ± 0.15
Shrubland	0.22 ± 0.15	0.21 ± 0.15	0.32 ± 0.14	0.31 ± 0.14	**0.42 ± 0.13**	0.42 ± 0.13	0.40 ± 0.13
Grassland	**0.24 ± 0.14**	0.06 ± 0.15	0.19 ± 0.15	0.19 ± 0.15	0.19 ± 0.15	0.19 ± 0.15	0.19 ± 0.15
Diversity (*H*)	0.15 ± 0.15	0.03 ± 0.15	0.24 ± 0.15	0.24 ± 0.15	0.25 ± 0.14	0.22 ± 0.14	0.17 ± 0.15
B. mesomelas							
Forest	0.26 ± 0.14	0.28 ± 0.14	0.34 ± 0.14	**0.36 ± 0.14**	0.26 ± 0.14	0.26 ± 0.14	0.26 ± 0.14
Shrubland	0.16 ± 0.15	0.07 ± 0.15	0.11 ± 0.15	0.06 ± 0.15	0.20 ± 0.15	**0.25 ± 0.14**	0.19 ± 0.15
Grassland	0.05 ± 0.15	0.00 ± 0.15	0.02 ± 0.15	0.01 ± 0.15	0.04 ± 0.15	0.05 ± 0.15	**0.06 ± 0.15**
Diversity (*H*)	0.01 ± 0.15	0.07 ± 0.15	**0.21 ± 0.15**	0.09 ± 0.15	0.03 ± 0.15	0.03 ± 0.15	0.02 ± 0.15
B. muscorum							
Forest	0.05 ± 0.15	0.03 ± 0.15	0.09 ± 0.15	0.02 ± 0.15	0.26 ± 0.14	**0.27 ± 0.14**	0.21 ± 0.15
Shrubland	0.05 ± 0.15	0.03 ± 0.15	0.09 ± 0.15	0.13 ± 0.15	0.17 ± 0.15	**0.20 ± 0.15**	0.17 ± 0.15
Grassland	0.06 ± 0.15	0.02 ± 0.15	0.08 ± 0.15	0.01 ± 0.15	0.02 ± 0.15	0.05 ± 0.15	**0.08 ± 0.15**
Diversity (*H*)	0.08 ± 0.15	0.02 ± 0.15	0.04 ± 0.15	**0.16 ± 0.15**	0.00 ± 0.15	0.01 ± 0.15	0.09 ± 0.15

APPENDIX 3.3. Continued.

	Spatial scale						
Species	**100m**	**250m**	**500m**	**750m**	**1500m**	**2000m**	**3000m**
B. pratorum							
Forest	0.16 ± 0.15	0.17 ± 0.15	0.26 ± 0.15	0.28 ± 0.15	0.34 ± 0.15	**0.36 ± 0.15**	0.33 ± 0.15
Shrubland	0.10 ± 0.15	0.18 ± 0.15	0.25 ± 0.15	0.27 ± 0.15	**0.32 ± 0.15**	0.29 ± 0.15	0.20 ± 0.15
Grassland	0.07 ± 0.15	0.04 ± 0.15	0.03 ± 0.15	0.02 ± 0.15	0.09 ± 0.15	0.07 ± 0.15	**0.15 ± 0.15**
Diversity (*H*)	0.05 ± 0.15	0.01 ± 0.15	0.01 ± 0.15	0.02 ± 0.15	0.04 ± 0.15	0.03 ± 0.15	**0.09 ± 0.15**
B. ruderarius							
Forest	0.06 ± 0.15	0.09 ± 0.15	0.00 ± 0.15	0.19 ± 0.15	0.21 ± 0.15	**0.25 ± 0.14**	0.01 ± 0.15
Shrubland	0.04 ± 0.15	0.26 ± 0.14	0.14 ± 0.15	0.16 ± 0.15	0.19 ± 0.15	0.26 ± 0.14	**0.51 ± 0.12**
Grassland	0.23 ± 0.15	0.11 ± 0.15	0.06 ± 0.15	0.11 ± 0.15	0.20 ± 0.15	0.28 ± 0.14	**0.36 ± 0.13**
Diversity (*H*)	0.19 ± 0.15	0.20 ± 0.15	0.12 ± 0.15	0.05 ± 0.15	0.04 ± 0.15	0.01 ± 0.15	**0.29 ± 0.15**
B. sichelii							
Forest	0.17 ± 0.15	0.27 ± 0.14	0.25 ± 0.14	0.29 ± 0.15	0.35 ± 0.13	0.30 ± 0.13	**0.44 ± 0.12**
Shrubland	0.13 ± 0.15	0.20 ± 0.15	0.17 ± 0.15	0.16 ± 0.15	0.25 ± 0.14	**0.30 ± 0.14**	0.25 ± 0.14
Grassland	0.10 ± 0.15	**0.15 ± 0.15**	0.10 ± 0.15	0.15 ± 0.15	0.10 ± 0.15	0.09 ± 0.15	0.07 ± 0.15
Diversity (*H*)	0.09 ± 0.15	0.09 ± 0.15	**0.11 ± 0.15**	0.02 ± 0.15	0.10 ± 0.15	0.05 ± 0.15	0.05 ± 0.15
B. soroeensis							
Forest	0.17 ± 0.15	0.18 ± 0.15	0.14 ± 0.15	0.13 ± 0.15	**0.18 ± 0.15**	0.15 ± 0.15	0.13 ± 0.15
Shrubland	**0.77 ± 0.06**	0.67 ± 0.09	0.23 ± 0.15	0.30 ± 0.14	0.09 ± 0.15	0.09 ± 0.15	0.05 ± 0.15
Grassland	**0.21 ± 0.15**	0.18 ± 0.15	0.18 ± 0.15	0.19 ± 0.15	0.11 ± 0.15	0.02 ± 0.15	0.03 ± 0.15
Diversity (*H*)	**0.07 ± 0.15**	0.04 ± 0.15	0.03 ± 0.15	0.07 ± 0.15	0.03 ± 0.15	0.02 ± 0.15	0.02 ± 0.15
B. terrestris							
Forest	0.20 ± 0.15	0.24 ± 0.15	**0.43 ± 0.13**	0.25 ± 0.14	0.24 ± 0.15	0.19 ± 0.15	0.37 ± 0.15
Shrubland	0.13 ± 0.15	0.10 ± 0.15	**0.28 ± 0.15**	0.27 ± 0.15	0.01 ± 0.15	0.05 ± 0.15	0.21 ± 0.15
Grassland	**0.32 ± 0.15**	0.19 ± 0.15	0.15 ± 0.15	0.06 ± 0.15	0.04 ± 0.15	0.09 ± 0.15	0.14 ± 0.15
Diversity (*H*)	0.24 ± 0.14	0.27 ± 0.14	0.13 ± 0.15	**0.41 ± 0.13**	0.25 ± 0.14	0.23 ± 0.14	0.09 ± 0.14
B. wurflenii							
Forest	0.06 ± 0.15	0.08 ± 0.15	0.00 ± 0.15	0.14 ± 0.15	0.03 ± 0.15	0.01 ± 0.15	**0.43 ± 0.13**
Shrubland	0.00 ± 0.15	0.02 ± 0.15	0.23 ± 0.15	0.08 ± 0.15	0.16 ± 0.15	0.28 ± 0.15	**0.73 ± 0.07**
Grassland	0.14 ± 0.15	0.17 ± 0.15	0.14 ± 0.15	0.04 ± 0.15	**0.23 ± 0.15**	0.30 ± 0.15	0.02 ± 0.15
Diversity (*H*)	0.09 ± 0.15	0.10 ± 0.15	0.20 ± 0.15	0.12 ± 0.15	0.30 ± 0.14	**0.40 ± 0.14**	0.38 ± 0.13

APPENDIX 3.4. Moran's *I* and significance (after sequential Bonferroni adjustment, $P < 0.05$) checking for spatial autocorrelation in the "best"-model residuals of both bumblebee species' presence and abundance.

	Presence		**Abundance**	
Species	**Moran I statistic**	***p*-value**	**Moran I statistic**	***p*-value**
B. hortorum	0.001	0.213	-0.007	0.274
B. humilis	-0.024	0.471	-0.001	0.235
B. jonellus	-0.008	0.308	-0.052	0.828
B. lapidarius	-0.077	0.950	0.001	0.108
B. lucorum	-0.032	0.570	-0.032	0.580
B. mesomelas	-0.026	0.508	-0.005	0.262
B. muscorum	-0.094	0.977	-0.083	0.969
B. pratorum	-0.029	0.537	-0.026	0.510
B. ruderarius	0.007	0.179	-0.012	0.341
B. sichelii	-0.036	0.614	-0.026	0.507
B. soroeensis	-0.018	0.402	-0.026	0.509
B. terrestris	-0.039	0.645	0.007	0.165
B. wurflenii	-0.002	0.242	-0.032	0.578

APPENDIX 3.5. Summarized results of Generalized Linear Models predicting the presence of bumblebees in both 1988-1989 and 2007-2009 study periods on the basis of topoclimatic (linear and quadratic temperature, precipitation, mean slope at 100 m and 3000 m buffer areas) and land-cover (forest, shrubland, grassland and the Shannon Diversity Index of land-cover, *H*) features. Results shown correspond to the best model (i.e., with the lowest corrected AICc), selected from combinations of variables presented in the table's heading line, at the fitted nested spatial scale of land-cover that maximized model predictability (see Appendix 3.2). Values shown include parameter estimates for the different variables and information-criterion scores (AICc) of best-fitted models. Only species present in at lest 25 localities in both study periods were included.

			Parameter estimates									
			Topoclimatic variables					**Land-cover variables**				**Goodness-of-fit**
Species	**Period**	**Intercept**	**Temp**	**Temp2**	**Rainfall**	**Slope 100**	**Slope 3000**	**Forest**	**Shrubland**	**Grassland**	***H***	**AICc**
B. hortorum	2007-2009	-3321.6	150.6	801.4	556.9	379.7	-	1220.7	1137.0	1634.5	-	20.64
	1988-1989	2.0	4.1	-	-	-	-	-	-	-	-	20.42
B. lucorum	2007-2009	473.8	-	1065.2	-1079.6	-	2195.0	-1810.9	-	-	5126.9	14.52
	1988-1989	0.2	-0.7	-	-	-	-	-	-	-	-	33.14
B. mesomelas	2007-2009	-134.9	-161.2	-	-	-	-79.2	-	-	-	-41.1	9.14
	1988-1989	-388.7	-763.4	-	-	95.1	-	-	-	-	-	6.66
B. muscorum	2007-2009	-0.6	0.6	-	-	-	-	-	-	-	-	32.05
	1988-1989	-687.2	95.7	-	-	90.2	-	253.6	286.9	304.8	-	14.54
B. pratorum	2007-2009	-0.3	-	-	-	-	-	-	-	-13.7	-	20.81
	1988-1989	21.7	-	370.5	-	-674.9	249.7	-	-496.2	-778.4	610.9	17.50
B. ruderarius	2007-2009	0.0	-3.1	-	1.8	-1.0	-	-	-1.6	-	-	28.14
	1988-1989	40.5	544.7	-496.0	-240.3	-	99.4	-	344.3	187.6	-129.8	20.64
B. sichelii	2007-2009	260.0	-820.5	-784.9	-	-	157.8	-501.6	-480.3	-	-	14.54
	1988-1989	-1510.6	-	-	-	-	395.1	-4941.9	-	-	1167.5	9.14
B. soroeensis	2007-2009	267.0	-456.4	-665.2	-	323.2	-	-	-	-	-	9.14
	1988-1989	-8.6	-5.1	-	1.2	-	-	3.9	2.8	2.8	-	29.60
B. terrestris	2007-2009	-0.6	-	-	-	1.3	-	-	-	2.2	-1.2	28.84
	1988-1989	-2.5	-	1.9	-	-	-	-	-	-	1.1	26.90
B. wurflenii	2007-2009	-80.6	-74.3	-	-85.7	-72.7	116.3	-142.5	-	-	-	14.54
	1988-1989	0.2	-	-	-	-	-	-	0.5	-	-	34.38

H = Shannon-Wiener diversity index of land-cover.

APPENDIX 3.6. Summarized results of Generalized Linear Models predicting the abundance of bumblebees in both historical (1988-1989) and current (2007-2009) study periods on the basis of topoclimatic and land-cover features. Results shown correspond to the best model (i.e., with the lowest corrected AICc), selected from a combination of variables presented in the table's heading line, at the fitted nested spatial scale that maximized model predictability (see Appendix 3.2). For further conventions, see Appendix 3.6.

			Parameter estimates									
			Topoclimate variables					**Habitat variables**				**Goodness-of-fit**
Species	**Period**	**Intercept**	**Temp**	**Temp2**	**Rainfall**	**Slope 100**	**Slope 3000**	**Forest**	**Shrubland**	**Grassland**	***H***	**AICc**
B. hortorum	2007-2009	-794820.1	-	1.0	-	0.8	-	349599.7	369869.5	339736.2	-	89.72
	1988-1989	-665539.1	-	-0.3	1.2	-	-0.7	292736.8	309709.1	284477.8	0.9	135.49
B. lucorum	2007-2009	0.8	-0.4	-	0.4	-	-	-	-	-	-	127.38
	1988-1989	-1.6	-0.4	0.4	-	-	-	0.9	1.0	-	-0.3	100.30
B. mesomelas	2007-2009	-3.7	-3.5	-	-	-	-	-	-	-	-	23.66
	1988-1989	-2.0	-1.1	-	-	0.5	-	-5.8	-	-	0.9	47.98
B. muscorum	2007-2009	-0.6	1.8	-1.2	-0.7	0.7	-	-	1.4	1.0	-0.7	56.66
	1988-1989	-6.8	11.1	-6.7	-6.4	-	5.3	-	-	4.2	3.9	33.86
B. pratorum	2007-2009	-48.9	-	-	-	-	-	50.2	50.1	-	2.4	31.89
	1988-1989	-3.6	-	-	0.7	-3.0	-	4.8	2.2	-	2.4	44.37
B. ruderarius	2007-2009	-1.1	-1.6	-	-	-	-1.2	-	-0.5	-	-0.9	70.09
	1988-1989	-1.4	2.7	-2.1	-1.7	-	1.4	-	3.3	1.7	-1.3	49.54
B. sichelii	2007-2009	-1.6	-5.0	-3.8	-0.9	-0.5	3.9	-3.3	-	-	0.8	77.86
	1988-1989	-4.8	-6.3	-4.2	-	-	3.1	-5.1	-	-	-	35.67
B. soroeensis	2007-2009	2.3	-2.1	-1.6	0.2	-	-	-0.8	-1.4	-1.2	0.6	168.81
	1988-1989	-0.4	-1.3	-	0.7	0.5	-0.5	-	-	-	-	73.65
B. terrestris	2007-2009	-0.5	-	0.5	-0.4	-	0.4	-	-	0.2	-0.7	80.53
	1988-1989	-0.9	-1.0	0.9	0.5	-0.7	-0.5	-	-1.3	-	-	86.27
B. wurflenii	2007-2009	-4.9	-7.8	-	-2.1	-5.8	-	-7.6	-6.1	-5.1	-	33.77
	1988-1989	2.6	-	-0.6	-0.5	-0.4	0.6	-2.3	-1.7	-2.1	-	98.61

APPENDIX 4.1. $\delta^{13}C$ and $\delta^{15}N$ isotopic values (mean ± SD) in pollen samples and trophic fractionation factors (Δ) for bumblebee samples at each site.

Site	***n***	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**Δ $\delta^{13}C$ (‰)**	**Δ $\delta^{15}N$ (‰)**
Prendes	19	-28.0 ± 0.66	-1.19 ± 1.29	1.71 ± 0.72	2.71 ± 1.69
Brañilín	20	-24.49 ± 1.11	1.62 ± 1.82	0.25 ± 0.68	-1.19 ± 1.98
Valle	8	-25.86 ± 0.87	0.60 ± 0.91	1.25 ± 0.40	1.67 ± 1.28
El Puerto	20	-25.08 ± 1.75	-0.64 ± 2.99	1.19 ± 0.53	2.71 ± 1.32
Gamoniteiru	9	-25.61 ± 0.66	0.26 ± 1.16	1.55 ± 0.86	1.79 ± 0.88
Peña Ubiña	19	-25.97 ± 0.42	0.28 ± 0.82	1.99 ± 0.66	1.06 ± 0.94

APPENDIX 4.2. Results of a linear mixed model (LMM) assessing the relationship between trophic niche width (SEA_c) of bumblebee species and (a) tongue length and (b) thorax width.

(a)

Fixed effects	**Estimate**	**SE**	***t* value**	**$MCMC_{mean}$**	**$HPD95_{lower}$**	**$HPD95_{upper}$**	**P_{MCMC}**	***t***
Intercept	-4.878	2.952	-1.653	-3.644	-11.783	4.035	0.334	0.116
Thorax width	1.082	0.548	1.974	0.828	-0.909	2.444	0.304	0.064
Tongue length	0.172	0.216	0.797	0.166	-0.310	0.664	0.477	0.436

Random effect	**Variance**	**SD**	**$MCMC_{mean}$**	**$HPD95_{lower}$**	**$HPD95_{upper}$**
Species	0.000	0.000	0.114	0.000	0.409
Site	1.275	1.129	0.632	0.213	1.175
Residual	0.342	0.585	0.884	0.550	1.279

(b)

Fixed effects	**Estimate**	**SE**	***t* value**	**$MCMC_{mean}$**	**$HPD95_{lower}$**	**$HPD95_{upper}$**	**P_{MCMC}**	***t***
Intercept	-3.955	2.702	-1.464	-3.445	-10.905	3.883	0.342	0.160
Thorax width	1.137	0.540	2.104	1.023	-0.441	2.534	0.174	**0.049**

Random effects	**Variance**	**SD**	**$MCMC_{mean}$**	**$HPD95_{lower}$**	**$HPD95_{upper}$**
Species	0.000	0.000	0.109	0.000	0.392
Site	1.210	1.100	0.630	0.240	1.153
Residual	0.339	0.582	0.856	0.548	1.248

APPENDIX 4.3. Results of a linear mixed model showing the relationship between % niche overlap and difference on tongue length between species pairs. $P < 0.05$ denoted in bold.

Fixed Effects	**Estimate**	**SE**	***t* value**	**$MCMC_{mean}$**	**$HPD95_{lower}$**	**$HPD95_{upper}$**	**P_{MCMC}**	***t***
Intercept	17.468	4.244	4.116	17.970	8.092	28.043	0.0082	0.0003
Tongue length	-9.478	4.028	-2.353	-10.110	-19.303	-1.656	**0.0260**	**0.0262**

Random effects	**Variance**	**SD**	**$MCMC_{mean}$**	**$HPD95_{lower}$**	**$HPD95_{upper}$**
Site	19.929	4.464	4.530	0.000	12.043
Residual	91.484	9.565	10.002	7.355	12.933

I hate myself, I hate clover, and I hate bees

Charles Darwin, from a letter to John Lubbock (1862)